# Respiration and panic

*DANIELA CALDIROLA*

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# RESPIRATION AND PANIC

## PROEFSCHRIFT

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*for my father*
*à moi, ma petite chérie*

*"...Sosteneva, fra l'altro, che le inopinate catastrofi non sono mai la conseguenza*
*o l'effetto che dir si voglia d'un unico motivo, d'una causa al singolare:*
*ma sono come un vortice, un punto di depressione ciclonica nella coscienza del mondo,*
*verso cui hanno cospirato tutta una molteplicità di causali convergenti.*
*Diceva anche nodo o groviglio, o garbuglio, o* gnommero,
*che alla romana vuol dire gomitolo...."*

*CARLO EMILIO GADDA,*
*Quer pasticciaccio brutto de via Merulana*

*"...He sustained, among other things, that unforeseen catastrophes*
*are never the consequence or the effect, if you will, of a single motive,*
*of a cause singular: nay, they are like a whirlpool, a point of cyclonic depression,*
*in the consciousness of the world, toward which a whole multiplicity*
*of converging causes have conspired. He also said knot or tangle,*
*or snarl, or* gnommero, *which in Roman dialect means skein...."*

*CARLO EMILIO GADDA,*
*That awful mess on via Merulana*

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# Chapter 1

# Introduction

## 1. Panic Disorder

According to DSM-IV, Panic Disorder (PD) is characterized by the occurrence of unexpected panic attacks. Panic attacks are brief periods of intense fear or discomfort with several somatic and / or psychological symptoms. At least four symptoms are required, having an abrupt development and reaching a peak within 10 minutes. The symptoms are:

1. Palpitations, pounding heart or accelerated heart rate
2. Sweating
3. Trembling or shaking
4. Sensation of shortness of breath or smothering
5. Feeling of choking
6. Chest pain or discomfort
7. Nausea or abdominal distress
8. Feeling dizzy, unsteady, lightheaded or faint
9. Derealization or depersonalization
10. Fear of losing control or going crazy
11. Fear of dying
12. Paresthesias
13. Chills or hot flushes

Since these symptoms could be present in different medical conditions or during use or withdrawal of several drugs, DSM-IV criteria require the exclusion of substance use or medical conditions. It should be noted, however, that the co-presence of PD and medical conditions, such as respiratory disorders, is frequent and often leads to incorrect diagnoses when the physicians attribute all the clinical symptoms to the medical conditons without identifying the presence of PD. Careful collections of the medical histories are crucial to avoid inaccurate diagnoses and incorrect therapies.
Three types of panic attacks are described. (1) Unexpected panic attacks, not associated

with situational triggers; (2) situationally bound panic attacks, occurring almost invariably on exposure to / in anticipation of situational triggers; (3) situationally predisposed panic attacks, more likely but not invariably associated with situational triggers. For the diagnosis of PD at least two unexpected panic attacks and the presence of anticipatory anxiety between the attacks are required. The anticipatory anxiety is defined by at least one of these conditions:
1. Persistent concern about having additional attacks
2. Worry about the implication of the attack or its consequences
3. Significant changes in behaviour related to the attacks

PD is often accompanied by Agoraphobia, the fear of being in places or situations from which escaping might be difficult or embarassing or in which help might not be available in the event of panic attack.
PD is a quite common disorder in the population. Lifetime prevalence rates range from 1.5 to 3.5% and it is about twice higher in women than in men. The peak of onset of PD is around the age of 25, but it varies considerably (25-35 years for women, 30-45 years for men) (Eaton et al, 1994; Katerndahl and Realini, 1993; Wittchen and Essau, 1993). PD has a high degree of comorbidity with other anxiety disorders, such as social phobia, generalized anxiety disorder, and obsessive-compulsive disorder (Goisman et al, 1994; Lepine and Lelouch, 1995), with major depression (Weissman et al, 1997) and with substance abuse (Regier et al, 1990). PD significantly affects the quality of life and has high social costs (Mendlowicz and Stein, 2000; Salvador Carulla et al, 1995).

## 2. Respiration and Panic Disorder

### The panic-respiration connection

Scientific research has shown that respiration is one of the main human functions involved in the phenomenology and the biological mechanisms of PD (Bellodi and Perna, 1998; Griez and Perna, 2003), leading to specific respiratory theories (Clark, 1986; Ley, 1985; Klein, 1993). Beyond the frequent observation of respiratory symptoms as core features of panic attacks, some important lines of experimental evidence support the existence of a panic-respiration connection.

(a) The association between hyperventilation and PD.

Patients with panic attacks and the "hyperventilation syndrome" show similar symptoms, including dyspnea, palpitations, tremors, paresthesias, faintness; the incidence of "hyperventilation syndrome" overlaps with panic disorder in up to 40% of patients (Garssen et al, 1983; Cowley and Roy-Byrne, 1987; de Ruiter, 1989). Despite these observations, the proposed causal role of hyperventilation in PD is doubtful. For example, hyperventilation challenge can induce some anxiety but not panic attacks in patients with PD (Gorman et al, 1984; Griez et al, 1988); chronic hyperventilation and hypocapnia are reported in less than 50% of the patients with PD (Gorman et al, 1986) and are not specific to PD as they were found in patients with other anxiety disorders (van den Hout, 1992). Finally, most of the studies show that hypercapnic challenge is more strongly panicogenic than hyperventilation (Antony et al, 1997; Gorman et al, 1994; Zandbergen et al, 1990) and the induced panic precedes hyperventilation and hypocapnia (Gorman et al, 1990). Overall, the experimental evidence suggests that hyperventilation is an important component of PD but a causal role seems unlikely.

(b) The association between respiratory diseases and PD.

Up to 40% of patients with PD have a childhood history of respiratory diseases, in particular asthma and bronchitis (Griez and Perna, 2003). This high childhood respiratory morbidity might be specific, since, to date, there are no reports of a higher than expected prevalence of other somatic disorders during childhood. On the other hand, there are higher than expected prevalence rates of PD in patients with Chronic Obstructive Pulmonary Disease (COPD) and an association between respiratory diseases and risk for panic attacks in the general population (Goodwin and Pine, 2002). Several studies have suggested an association between panic disorder and asthma (Carr, 1999; Davies et al, 2001; Perna et al, 1997). Others have shown a higher prevalence of PD (8%-24%) in patients with COPD compared to controls (Karajgi et al, 1990). In addition, the prevalence of respiratory diseases in patients with PD is higher than in healthy controls and in patients with other psychiatric disorders (obsessive compulsive, depressive and eating disorders) (Perna et al, 1994; Verburg et al, 1995). We have proposed that the presence of a respiratory disease increases the risk of developing panic symptoms in subjects at risk for panic disorder (Perna et al, 1997).

(c) The hyperreactivity to hypercapnia in PD.

Patients with PD are hyperreactive to the inhalation of hypercapnic gas mixtures. Regardless of the method of administration (continuous breathing of 5% or 7%$CO_2$ gas mixture / single vital-capacity inhalation of 35% $CO_2$-65% $O_2$ gas mixture), hypercapnia provokes a panic-like reaction in subjects with PD, whereas in healthy controls it does not (Gorman et al, 1984; Griez et al, 1987). Patients with obsessive-compulsive disorder, generalized anxiety disorder and animal specific phobia, all showing comparable levels of anticipatory anxiety and arousal, failed to show any significant anxiety reaction to 35% $CO_2$inhalation. Patients with major depression or eating disorder did not react to $CO_2$ inhalation as well. $CO_2$ vulnerability is considered an indication of specific mechanisms underlying PD. Patients with situational phobia, subjects with sporadic panic attacks and, at least in part, patients with social phobia have $CO_2$ induced reactions similar to those of patients with PD. $CO_2$ hypersensitivity might be a biological marker of vulnerability for a "panic-phobic spectrum", whose central clinical phenomenon might be the panic attack (for review see Bellodi and Perna, 1998; Griez and Perna, 2003; Verburg et al, 2001).

Genetic relationships between panic and respiratory vulnerability have been studied as well. A familial association between PD and hypersensitivity to hypercapnia was found (Coryell, 1997; van Beek and Griez, 2000; Perna et al, 1995; Perna et al, 1996). $CO_2$ vulnerability and respiratory symptoms might be associated with a subtype of PD ("respiratory PD") that is specifically related to a greater familial loading (Horwath et al, 1997; Perna et al, 1996).

Finally, preliminary evidence shows that hypoxia has panicogenic properties in patients with PD as well, in contrast with healthy controls (Beck et al, 1999). These findings indicate that patients with PD might be hypersensitive to different stimuli that influence respiration. This idea is consistent with the findings of chaotic and irregular baseline breathing patterns in patients with PD (see below): baseline instability in the respiratory function may affect the ability of patients with PD to maintain an adequate respiratory homeostasis when different stimuli occur. Since hypoxia induces ventilatory response to restore respiratory homeostasis, the baseline respiratory irregularity might constrain efficient compensatory mechanisms during the hypoxic challenge, influencing the occurrence of the induced panic attacks. Therefore, PD might be associated with a global malfunction of the respiratory system rather than a specific abnormal chemosensitivity for $CO_2$. Further studies with different respiratory manipulations are necessary to clarify this issue.

(d) The respiratory physiology in PD.

Studies investigating baseline respiratory physiology in patients with PD have reported mixed results.

Most of the studies reporting data on mean baseline measures of respiratory physiology (respiratory rate, tidal volume, minute ventilation and respiratory gases partial pressures) did not find significant differences between patients with PD and healthy controls or patients with other anxiety disorders (for review see Bellodi and Perna, 1998; Griez and Perna, 2003); This has led many researchers to question the central role of respiration in the etiopathogenesis of PD (see below). However, these findings may arise from improper methods in measuring respiratory parameters. Recently, when respiratory tracings over time rather than the average values of the respiratory parameters were studied, more consistent and specific respiratory abnormalities were found. Patients with PD showed an increased respiratory irregularity, attributed to frequent sighs, than healthy controls and patients with generalized anxiety disorder. The tidal volume irregularity persisted after both doxapram-induced hyperventilation and cognitive intervention, suggesting that it might be an intrinsic and stable feature of patients with PD (Abelson et al, 2000; Abelson et al, 2001; Wilhelm et al, 2001). Finally, recent studies showed a higher variability in response to 5% $CO_2$ inhalation in healthy relatives of patients with PD compared to relatives of healthy controls and of patients with affective disorders, and more irregularities in the respiratory rate of children with childhood anxiety disorders who developed panic symptoms after $CO_2$ inhalation (Coryell et al, 2001; Pine et al, 2000).

## Theories on PD and the panic- respiration connection

(a) The False Suffocation Alarm Theory

The main theory on the panic-respiration connection was developed by Donald Klein (Klein, 1993). He considers the panic attack an indication of a pathologically sensitive suffocation alarm system, that does not overlap with simple fear reactions. He provides several arguments to support his theory. The existence of an inborn suffocation monitor could be supported by the existence of the congenital central hypoventilation syndrome in which the chemical suffocation monitor is lacking, leading to cessation of breathing when the patients fall asleep.

Panic attacks are often characterized by the prominence of respiratory symptoms, whereas, in frightening situations, sweating and pounding heart are the most prominent symptoms. Panic attacks are also different from acute anxiety and fear reactions since there is no activation of the hypothalamic-pituitary-adrenal (HPA) axis. The False Suffocation Alarm theory also explains the panicogenic effects of lactate and carbon dioxide administration to patients with panic disorder. If an abnormal suffocation alarm system exists, altered chemosensitivity might be expected. Studies on the ventilatory response to $CO_2$ have yielded contradictory results (Gorman et al, 1988; Papp et al, 1995). A recent study by Katzman and co-workers did not show significant differences in chemoreflex sensitivity or threshold, suggesting that the triggering of the putative false suffocation alarm may not be implemented within the respiratory chemoreflexes (Katzman et al, 2002). Till now the issue of the respiratory chemosensitivity in PD remains unclear and requires clarification by future methodological studies. The lack of clear evidence of abnormal chemosensitivity and abnormalities in the mean baseline measures of respiratory physiology in patients with PD have led to criticism and alternative explanations of the panic-respiration connection.

(b) Alternative Theories

Ronald Ley (1985) proposed a "dyspnea/suffocation fear" theory. He proposed that the experience of acute severe dyspnea accompanied by suffocation fear are prerequisites for the classic primary panic attacks. The experience of dyspnea underlying the primary panic attacks ("out-of-the blue") could arise from stress-induced respiratory responses. The secondary panic attacks (situationally bound / predisposed) could be linked to Pavlovian/classical conditioning of dyspnea/suffocation fear. He argues that the probability that a single panic attack could lead to PD depends on the intensity and duration of the initial attack and whether or not the accompanying environmental cues facilitate generalization of dyspnea/suffocation fear to a relatively broad range of stimuli.

Clark (1986) proposed a "cognitive theory" of panic. He proposed that panic attacks arise from the catastrophic misinterpretation of harmless bodily sensations. Thus, a person might misinterpret palpitations as impending cardiac arrest, dizziness as impending collapse, or de-realization as impending insanity. By a positive feedback loop, catastrophic misinterpretation increases anxiety and intensifies bodily sensations until they culminate in panic.

Recently, Gorman and coworkers (2000) proposed that that respiration does not play a specific role in PD and that $CO_2$ inhalation might be a non-specific trigger of panic attacks. $CO_2$ could provoke panic by stimulating a hypersensitive "fear network". Heightened amygdalar activity could lead to abnormal response, i.e. the panic attack, to non-specific stimuli, such as physiological somatic cues and sensory information. Overall, these alternative theories of PD consider the panic attack a fear reaction. However the main criticism is that this idea is not supported by consistent biological findings. To date, the data about the activation of the HPA axis during the panic attacks have been controversial but most of the studies have shown a lack of significant activation of HPA in subjects with PD. Panicogenic agents such as sodium lactate and carbon dioxide are not accompanied by activation of HPA whereas other agents such as fenfluramine, yohimbine, metachlorophenylpiperazine seem characterized by an involvement of HPA. The former group of agents provokes panic attacks with prominent respiratory symptoms whereas the latter does not (Hollander et al, 1989; Seir et al, 1997; Sinha et al, 1999). Recently, Bailey and coworkers found an increase of subjective fear and an activation of the HPA axis during a single inhalation of 35% $CO_2$ in a sample of healthy subjects. However, the mechanisms underlying the reaction to $CO_2$ challenge are not necessarily the same in healthy subjects and in subjects with PD; furthermore none of the subjects in the study panicked with 35% $CO_2$. Thus, the observed neuroendocrinological responses might be different than those of the panic attacks. In conclusion, it seems unlikely that panic attacks, particularly those characterized by marked respiratory symptoms, are simply equivalent to fear/stress reactions. Thus, in PD specific anatomical circuits, not completely overlapping with the "fear network" circuits, could be involved. Mechanisms linked to the "fear network" circuits might underly other features of PD such as anticipatory anxiety and avoidant behaviours.

## Respiration and Panic Disorder: Looking at complexity

Physiological functions, such as respiration or cardiac function, are characterized by dynamic processes with complex interactions between multiple inputs. The moment (i.e. mean and standard deviation) and linear statistics are considered inappropriate for the analyses of physiological signals. Respiratory signals usually represent the output of complex mechani-

sms, including multiple feedback/coupling interactions and inputs from internal and external sources. The best way to determine respiratory abnormalities might be an analysis of the complexity of the respiratory tracing over time, by employing non-linear statistic methods that are considered the gold standard for the study of physiological functions (Pincus, 1991). Using this approach, consistent and specific abnormalites in respiratory patterns of patients with PD can be found. Chaotic baseline respiratory patterns in patients with PD (Yeragani et al, 2002) and in children of patients with PD (Perna et al, 2002) were found.
The focus of this thesis is the experimental study of respiratory function in PD and its implication in pathophysiology and therapy of the disorder.

## General overview

Chapter 1 provides a brief introduction of PD and an overview of the main lines of evidence supporting the existence of a panic-respiration connection (see above).
Several studies, performed by the group of Prof. Griez in Maastricht and our group, have confirmed the 35% $CO_2$ challenge as a valid experimental model of panic with good clinical validity, replicability, symptom convergence and specificity (Uhde and Tancer, 1990). In Chapter 2 we describe the presence of hypersensitivity to 35% $CO_2$ in patients with PD and Social Phobia (SP). The aim of the study was to test whether PD and SP might be two clinical syndromes that share common underlying pathogenetic mechanisms resulting in similar vulnerability to $CO_2$ inhalation. Our results suggest that PD and SP share a common hypersensitivity to $CO_2$and thus might belong to the same spectrum of "respiratory" vulnerability.
Drugs effective in the treatment of PD reduce hyperreactivity to 35% $CO_2$ in patients with PD, whereas ineffective ones are unable to block it. This reduction might be an indication of the normalization of pathophysiological mechanisms underlying PD (Bellodi and Perna, 1998). In the study in Chapter 3 we investigated the effect of modulation of serotonergic / noradrenergic systems on $CO_2$ reactivity. Our results indicate that the modulation of the serotonergic system is more relevant for $CO_2$ hyperreactivity than the noradrenergic one. We discuss the idea that the anti-panic effect of serotonergic anti-panic drugs could be explained by their effect on the respiratory system.
Despite the evidence for a connection between panic attacks and respiration, the nature of

respiratory abnormalities remains unclear. Previous studies measuring the mean values of baseline respiratory parameters found discordant results whereas a greater irregularity in breathing patterns was found and it has been attributed to frequent sighs. In order to clarify the nature of respiratory abnormalities in PD, in the study in **Chapter 4** we investigated the possible differences in the breath by breath complexity of respiration dynamics in patients with PD and healthy subjects. We applied a non-linear statistic measure, the Approximate Entropy index (ApEn), since non-linear methods are considered the gold standard to measure the complexity of physiological functions such as respiration. Patients with PD showed higher entropy in respiratory baseline patterns, indicating higher levels of irregularity and complexity in their respiratory function. Sighs do not fully explain the irregularity of breathing patterns. Higher respiratory entropy might represent a vulnerability factor to panic attacks.

The question of whether the behavioural reactivity to $CO_2$ is related to an abnormal respiratory function is still debated. In the study in **Chapter 5** we investigated if higher respiratory irregularity might explain the behavioural reactivity to hypercapnic stimulation. We found that the patients who panicked during 35% $CO_2$ challenge have higher respiratory irregularity than patients who did not panic. Moreover, the higher irregularity of tidal volume is a respiratory predictor of panic response to $CO_2$ inhalation. Our results suggest that chaotic breathing might influence the occurrence of the induced panic attacks, supporting the idea of an abnormal respiratory function in PD.

In the study in **Chapter 6** we investigated the type of dyspnea induced by 35% $CO_2$ challenge in patients with PD since it might suggest possible ideas on the neurophysiological pathways involved in the panic-respiration connection. We found that the sense of Breathing Effort is the most peculiar dyspnea sensation in $CO_2$-provoked panic attacks while the Sense of Suffocation seems to be less relevant. The dissociation between the increased central respiratory command and the decreased efficiency of the respiratory response in patients with PD might underlie the Breathing Effort during the $CO_2$ challenge. The Sense of Suffocation, linked to chemosensitivity, is involved in $CO_2$ reactivity but it might not be central to unexpected panic attacks.

Daily smoking is associated with an increased risk for later occurrence of panic attacks, possibly by impairing respiratory system function. The aim of our study in **Chapter 7** is to investigate possible mechanisms linking smoking and respiratory function in PD. We found

that smoking is associated with a higher irregularity in the baseline respiratory patterns in patients with PD, whereas in healthy subjects is not. The finding that non-smoking patients also have a higher respiratory irregularity than healthy subjects supports the idea that the respiratory irregularity might be an intrinsic feature of patients with PD. Smoking might act as "disruptive" factor specific to the baseline respiratory instability of patients with PD, possibly influencing the onset and/or the maintenance of the disorder.

Finally, in the study in Chapter 8, in order to test the idea that anti-panic properties of serotonergic drugs could be linked to their effect on the respiratory system, we evaluated the influence of one week paroxetine treatment on baseline respiratory patterns in PD. We found that paroxetine significantly decreases the irregularity and "disorder" in respiratory patterns of patients with PD. This supports the idea that a modulation of respiratory function by the serotonergic system might be an important mechanism of the anti-panic drugs efficacy. The decrease of respiratory irregularity might indicate a "normalization" of the abnormal respiratory function underlying PD.

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# Chapter 2

## The 35% $CO_2$ challenge test in Panic Disorder and Social Phobia

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## Abstract

Panic Disorder (PD) and Social Phobia (SP) share many clinical, demographic and biological characteristics. To investigate the relationships between the two disorders, the responses to inhalation of 35% carbon dioxide ($CO_2$) and 65% oxygen ($O_2$) gas mixture were assessed. Sixteen patients with PD, 16 patients with SP, 13 patients with both SP and PD, 7 patients with SP who experienced sporadic unexpected panic attacks and 16 healthy controls inhaled one vital capacity of 35% $CO_2$ or of compressed air. A double blind, random, cross over design was used. PD patients and SP patients showed similar anxiogenic reactions to 35% $CO_2$, both stronger than controls. Patients with both disorders and SP patients with sporadic unexpected panic attacks reacted like subjects with PD or SP alone. These results suggest that PD and SP share a common hypersensitivity to $CO_2$ and thus might belong to the same spectrum of vulnerability.

## Introduction

Social Phobia (SP) and Panic Disorder (PD) are both included in the anxiety disorders section of DSM IV (APA, 1994). Several studies showed that PD and SP share many clinical, demographic and biological characteristics. According to DSM IV, they are both characterised by panic attacks, although in PD they are often unexpected while in SP they are almost invariably situational, with anticipatory anxiety and with phobic avoidance, and the usual course of both disorders is chronic (APA, 1994; Liebowitz et al., 1985). From an epidemiological point of view, both are characterised by young age at onset and by higher prevalence in female subjects (APA, 1994). In addition, it has been reported that PD and SP have a high rate of comorbidity (APA, 1994; Liebowitz et al., 1985; Weissman, 1988; Lepine et Lelouch, 1995). Similar therapeutic responses have been observed in social phobics and patients with PD to treatment with mono-amine oxidase inhibitors (Van Vliet et al., 1995; Versiani et al., 1992; Gorman et al., 1985; Deltito and Stam al., 1989), high potency benzodiazepines (Spier et

al., 1986; Ballanger et al., 1990; Davidson et al., 1990; Muniack et al., 1990), cognitive-behavioral therapy (Beck et al., 1992; Liebowitz et al., 1985) and, recently, treatment with selective reuptake inhibitors (Sternbach et al., 1990; Black et al., 1992; Schneier et al., 1992; Van Amerigen et al., 1993a, 1993b; Denis et al., 1995; Den Boer et al., 1987; Gorman et al., 1987; Schneier et al., 1990; Hoen-Saric et al., 1993).

In recent years, data from the literature have clearly demonstrated that inhalations of gas mixtures of 35% $CO_2$ and 65% $O_2$ have a panicogenic effect in patients with PD, with good sensitivity (Griez et al., 1987; Fyer et al., 1987; Griez et al., 1990b; Gorman et al., 1994; Papp et al., 1993a; Van den Hout et Griez, 1984; Perna et al., 1994; Perna et al., 1995c) and specificity. Patients with Generalized Anxiety Disorder, Obsessive-Compulsive Disorder, Animal Simple Phobia and Mood Disorders react like healthy controls (Griez et al., 1990a; Gorman et al., 1990; Papp et al., 1993b; Perna et al., 1995b, 1995a; Verburg et al., 1994; Verburg et al.,1995). It has been hypothesized that in patients with PD the threshold of sensitivity for $CO_2$ in central chemoreceptors is lower than in normals. Carbon dioxide might trigger a "false suffocation alarm" provoking an intense autonomic and anxiety reaction (Klein, 1993; Klein, 1994).

Only two studies have investigated hypersensitivity to 35% $CO_2$ in social phobics, reporting rates of $CO_2$-induced panic higher those found in healthy controls and lower than those in patients with PD (Gorman, 1990; Papp, 1993b). Results from a preliminary study partially confirmed these data, showing that patients with social phobia react to one inhalation of 35% $CO_2$ like patients with panic disorder (Caldirola et al., 1995).

In the present study, we assessed the presence of hypersensitivity to 35% $CO_2$ in social phobic patients with and without sporadic unexpected panic attacks (SPA), in patients with PD and in patients with both PD and SP to examine the relationships between these two disorders. The main goal was to see whether PD and SP might be two clinical syndromes that share common underlying pathogenetic mechanism expressed by similar vulnerability to $CO_2$ inhalation.

## Methods

### Subjects

Five groups of subjects were included in this study: 16 patients with PD, 16 patients with SP, 13 patients with both SP and PD, 7 patients with SP and sporadic unexpected panic attacks and 16 healthy controls. Patients with Social Phobia with/without PD or sporadic unexpected panic attacks were seen consecutively over 2 years, patients with "pure" PD were consecutively recruited over 4 months at the Anxiety Disorders Clinical and Research Unit at the Department of Neuropsychiatric Sciences of S.Raffaele Hospital, Milan. Controls were recruited by advertisements placed around the University. Diagnoses were made by the Diagnostic Interview Schedule, Version III-R (DIS-R; Robins et al., 1989); interviewers were psychiatrists or residents in psychiatry trained in the use of the DIS-R interview. Data obtained were reanalyzed according to DSM IV criteria, and consensus diagnoses were made by two experienced psychiatrists.

The severity of phobic symptoms at the time of the challenge was assessed by the Fear Questionnaire (FQ) (range 0-120), a self-rating scale composed of three sub-scales scoring agoraphobia (FQ-AGO), social phobia (FQ-FS) and blood injury phobia (FQ-BI) (Marks and Mathews, 1979). The severity of panic symptoms was evaluated by the number of spontaneous panic attacks in the last month and by the severity of agoraphobic avoidance according to DSM IV classification.

Controls had never fit any lifetime psychiatric diagnoses, according to the DIS-R, and had never experienced unexpected panic attacks.

Exclusion criteria for all subjects were significant cardiocirculatory and respiratory disorders, personal or family history of cerebral aneurysm, significant hypertension (systolic > 180 mmHg, diastolic > 100 mmHg), pregnancy or epilepsy, all according to direct physical examination and to careful collection of medical histories. Other exclusion criteria for all the patients were the presence of all lifetime and current psychiatric disorders, including substance abuse or dependence and situational specific phobias, other than the ones described.

At the time of the challenge test, all subjects had to have not taken any psychotropic medications during the last 2 weeks. They were asked to refrain from alcohol for at least 36 hours, beverages containing xanthine for at least 8 hours and food or smoking for at least 2 hours preceding the test.

All the participants gave their informed consent to the study after a detailed explanation of the procedure.

## Apparatus

Two different gas mixtures were employed: compressed air (placebo) and a mixture of 35% $CO_2$ and 65% $O_2$. Both gases were inhaled through the same self-administration mask. Vital capacity was evaluated by a respirometer (Wright respirometer Mark 20, Ferraris Medical Limited) connected to the self administration mask. The same respirometer measured the gas volume delivered in each inhalation.

## Procedure

All subjects were tested in a double-blind, random, cross-over design, according to the method described elsewhere (Perna et al., 1995a). Subjects were informed they would be inhaling two harmless gas mixtures containing different percentages of $CO_2$ and $O_2$, and they might experience some discomfort, ranging from a few neurovegetative symptoms to a definite sensation of anxiety, but the possibility of a panic attack was not mentioned, according to Griez et al. (1987) procedure, to avoid any negative cognitive bias related to expectation. Although 35% $CO_2$/65% $O_2$ gas mixture has a distinct "taste", it is unlikely that patients might be able to link this taste with high or low concentration of $CO_2$. The brevity (few seconds-one minute) of the panic reaction induced by 35% $CO_2$ inhalation, which completely disappears in few minutes, justifies the latter decision ethically. Vital capacity was measured and baseline anxiety assessed by the State-Trait Anxiety Inventory for state anxiety (STAI-1)(Spielberger, 1970) and then each subject inhaled one vital capacity of 35% $CO_2$-65% $O_2$ or of compressed air, in a randomly assigned order, at an interval of 25-30 minutes. At the end of each inhalation subjects were asked to hold their breaths for 4 seconds. The test was considered valid only if the subject had inhaled at least 80% of the previously measured vital capacity.
Immediately before and after each inhalation (Air or $CO_2$) anxiety was evaluated by the Panic Symptom List (PSL III-R) (Pols et., 1991), a self-rating questionnaire assessing the 13 panic symptoms described in DSM III-R, on a 5 point scale (0= absent, 1= mild, 2= moderate, 3= severe, 4= very intense) and a Visual Analogue Scale for Anxiety (VAS-A) describing the degree of global subjective anxiety on a continuum from 0 (no anxiety present) to 100 (the worst anxiety ever imaginable).

### Assessment of $CO_2$ reactivity

*Quantitative assessment*

The reactivity to 35% $CO_2$ inhalations was evaluated as Δ% VAS-A (the percentage of maximum increment or decrement possible on the VAS-A scale) (Perna et al, 1995b), calculated as follows:

a. if Δ VAS-A (post-$CO_2$ VAS-A values minus pre-$CO_2$ VAS-A values) was positive, then Δ% VAS-A = ΔVAS-A x 100/(100- VAS-A before $CO_2$).

b. if ΔVAS-A was negative, then Δ% VAS-A = ΔVAS-A x 100/VAS-A before $CO_2$.

*Qualitative assessment*

According to the ideal threshold obtained by Receiver Operating Characteristic (ROC) analysis of the 35% $CO_2$ challenge (Battaglia & Perna, 1995), the reaction was considered "positive" if Δ% VAS-A > 26 and "negative" if Δ% VAS-A < 26.

The reaction to the 35% $CO_2$ challenge was considered a provoked panic attack, when it included all four of the following criteria: a "positive" reaction; a sensation of fear or panic; at least 4 symptoms among those described in DSM III-R, including at least one of the DSM III-R cognitive symptoms (fear of dying, going crazy or losing control). Social phobics were also asked to compare the reaction to $CO_2$ with anxiety induced by social situations.

### Data Analyses

To assess the significances of any differences in continuously distributed variables in the five groups, Analysis of Variance (ANOVA) and the post-hoc Student t-test with Bonferroni's correction were applied. Chi-square analyses were applied to compare the proportions of positive reactions, $CO_2$-induced panic attacks and sex distributions in the five groups. When two groups were compared, the Student t-test or the chi-square analysis with Bonferroni's correction was applied where appropriate. Pearson's correlation was applied to evaluate the relationships between STAI-1 scores and VAS-A before $CO_2$ or Δ% VAS-A among patients.

$CO_2$ reactivity was evaluated quantitatively as Δ% VAS-A (post-gas values minus pre-gas values), by a multivariate ANOVA in which "Procedure" (P)($CO_2$ vs Air) was treated as a repeated measures factor, "Diagnosis" (D) (PD vs SP vs SP/PD vs SP/SPA vs CT) was the grouping factor and Δ% VAS-A scores were the dependent variables.

## Results

Clinical and demographic characteristics of the study groups are listed in table 1.

The five groups did not differ significantly in sex or age. The mean age of onset for PD and SP of SP/PD patients did not differ from those of PD and SP patients.

The patterns of distribution for agoraphobia between SP/PD and PD patients did not differ significantly. SP/PD patients had had similar numbers of spontaneous panic attacks in the month preceding the challenge as the PD patients. There were significant differences for FQ-FS scores in the five groups ($F= 22.9$; $df= 4, 63$; $p<0.001$). Post-hoc comparisons showed similar scores for SP, SP/PD and SP/SPA patients, but significantly higher than for PD patients and controls.

Baseline anxiety, expressed by STAI-1 scores and VAS-A before $CO_2$, $\Delta$% VAS-A after 35% $CO_2$ and air and the rate of positive reactions and of induced-panic attacks in the five groups are reported in table 2. ANOVA showed a significant "Diagnosis" effect for STAI-1 scores ($F= 8.6$; $df= 4, 63$, $p< 0.001$) and VAS-A before $CO_2$ ($F= 4.1$; $df= 4, 63$, $p< 0.005$). Post-hoc comparisons showed significantly different STAI-1 scores in patients with PD, SP, SP/PD, SP/SPA than in controls while the patient groups did not differ from each other. Post-hoc comparisons showed significantly higher VAS-A before $CO_2$ in patients with SP/PD than in controls and patients with SP, while no differences were found comparing patients with SP and those with PD. There were no significant differences for STAI-1 scores comparing positive with negative reactors and panickers with non-panickers. There was a significant correlation between STAI-1 scores and VAS-A before $CO_2$ ($r=.49$, $p<0.0001$). No correlation was found between STAI-1 scores and $\Delta$% VAS-A in the patient groups.

MANOVA showed significant Diagnosis by Procedure interaction ($F= 2.6$, $df= 4, 63$; $p< 0.05$) for $\Delta$% VAS-A. Post-hoc comparisons showed similar reactions to 35% $CO_2$ in PD, SP, SP/PD and SP/SPA patients, all stronger than in controls.

Patterns of distribution for rates of positive reactions ($\chi 2= 19.7$, $df= 4$, $p<0.001$) and induced-panic attacks ($\chi 2= 14.6$, $df= 4$, $p< 0.001$) were different in the five groups. Post-hoc comparisons showed similar rates of provoked panic and positive reactions in PD, SP, SP/PD, SP/SPA patients. Rates of positive reactions were significantly higher in patients' than in controls. Rates of induced-panic attacks were higher in patients groups than in controls, significantly for PD, PD/SP, SP.

There were no significant differences for positive responses and induced-panic attacks to air-placebo for the five groups.

## Discussion

The results of our study show that patients with SP have a very similar reaction to the 35% $CO_2$ test as patients with PD, and stronger than that in controls, confirming our preliminary results (Caldirola et al., 1995). Patients with both disorders and SP patients with sporadic unexpected panic attacks react like subjects with PD or SP alone. The presence of similar strong reactions across all social phobics groups, from those with the co-occurrence of PD to those with "pure", support the idea that $CO_2$ hypersensitivity is related to Social Phobia "per se".
Previous publications reported that the rate of 35% $CO_2$-induced panic among patients with SP were not significantly different from those of patients with PD (Gorman et al., 1990) or in the midway between the rates for PD patients and normal subjects (Papp et al., 1993b). In addition, Gorman et al. (1988) reported that all of three social phobics reacted with a panic attack to prolonged inhalation of 7% $CO_2$. Our findings are in line with these data although not completely comparable for the different methods applied. The reaction to 35% $CO_2$ observed in SP patients cannot be due to performance-induced anxiety, since the positive responses to air-placebo are not statistically significant and $CO_2$-induced panics were reported to be different from anxiety induced by social situations. Although the relatively low severity of agoraphobia in our patients with PD may have contributed to mask the differences between social phobics and patients with PD, this factor seems unlikely to explain our results since we have previously observed no significant relationship between the severity of agoraphobia and 35% $CO_2$ reactivity (Perna et al., 1994).
In the subgroup of social phobics hypersensitive to $CO_2$ there might exist a deranged "suffocation alarm monitor" similarly to patients with PD. These two groups of patients might share at least some biological and/or psychological pathogenetic mechanisms.
Since two recent studies have reported hypersensitivity to 35% $CO_2$ in situational simple phobics (Verburg et al., 1994) and subjects with sporadic unexpected panic attacks (Perna et al., 1995c), we can think that $CO_2$ hypersensitivity might be a biological marker of vulnerability for a "Panic-Phobic Spectrum", whose central clinical phenomenon might be panic attacks (Perna et al., 1995c) separate from GAD, OCD and animal simple phobia, anxiety disorders in

which sensitivity to 35% $CO_2$ is normal. An isolated unexpected panic attack, not identified clinically or not recognized by the patient, might induce the development in some subjects of anticipatory anxiety for social or specific situations and avoidant behaviours, leading to SP or Situational Simple Phobias. Alternatively, social or situational simple phobics hypersensitive to $CO_2$ might be predisposed to develop panic attacks in the future. Finally, $CO_2$ hypersensitivity might be related not only to unexpected but to all types of panic attacks and thus also for social phobia, the triggering phenomena might be represented by panic attacks.

To test this hypothesis, it might be useful to see whether relatives of social phobics have a significantly increased risk for PD and relatives of patients with PD for SP. Although some studies have indicated that relatives of social phobics have an increased risk for SP but not for other anxiety disorders (Fyer et al., 1993) and that the rate of SP among relatives of patients with PD is not significantly higher than the rate in relatives of controls (Reich and Yates, 1988), recent data report an increased rate of SP in the families of PD patients (Weissman et al., 1993; Horwath et al 1995) thus suggesting that common pathogenetic mechanisms in both panic disorder and social phobia might exist.

Table 1. Demographic and clinical characteristics of the sample.

| | CT n=16 | SP n=16 | SP/PD n=13 | SP/SPA n=7 | PD n=16 |
|---|---|---|---|---|---|
| Age (yrs) | 25.8±2.8 | 30.7±9.6 | 28.7±8.9 | 26.3±8.9 | 31.7±6.4 |
| Sex (males) | 11 (69%) | 6 (37%) | 5 (38%) | 4 (57%) | 8 (50%) |
| Age at onset (yrs) | | | | | |
| - Panic attacks | | | 21.9±5.2 | 23.0±9.4 | 27.3±6.8 |
| - Social Phobia | | 18.7±8.7 | 15.1±5.7 | 20.0±9.7 | |
| Onset: | | | | | |
| - SP before SPA | | | 10 (70%) | 5 (71%) | |
| - SPA before SP | | | 3 (30%) | 2 (29%) | |
| N° of spontaneous panic attacks a week in the last month | 0 | 0 | 1.4±1.9 | 0 | 1.4±2.5 |
| Agoraphobia | | | | | |
| - none | | | 5 (38%) | 6 (86%) | 4 (25%) |
| - mild | | | 3 (23%) | 1 (14%) | 7 (44%) |
| - moderate | | | 4 (31%) | 0 (0%) | 3 (19%) |
| - severe | | | 1 (8%) | 0 (0%) | 2 (12%) |
| Fear Questionnaire: | | | | | |
| - FQ-AGO | 1.5±2.7 | 7.1±7.8 | 14.2±10.7 | 10.6±9.2 | 12.7±7.2 |
| - FQ-FS | 5.2±4.6 | 26.8±7.9 | 24.9±11.4 | 25.0±11.9 | 8.7±6.1 |
| - FQ-BI | 9.4±7.4 | 14.8±9.5 | 17.2±11.8 | 12.2±3.3 | 15.7±10.5 |

Age, age at onset, n° of panic attacks and FQ scores are expressed as means ± SD
SP: social phobia, SPA: sporadic unexpected panic attacks, PD: panic disorder, CT: healthy controls

Table 2. Baseline anxiety and reactivity to 35% $CO_2$ of patients with SP, SP/PD, SP/SPA, PD and healthy controls.

| | CT n=16 | SP n=16 | SP/PD n=13 | SP/SPA n=7 | PD n=16 |
|---|---|---|---|---|---|
| STAI-1 score | 34.4±8.8 | 48.3±12.9 | 28.7±8.9 | 50.6±12.7 | 41.3±9.9 |
| VAS-A before $CO_2$ | 15.9±14.5 | 19.7±17.3 | 42.1±24.1 | 24.6±19.7 | 22.6±17.7 |
| Δ% VAS-A: | | | | | |
| - after $CO_2$ | 7.8±32.8 | 61.3±33.3 | 57.4±36.1 | 57.3±40.5 | 54.6±37.4 |
| - after Air | -35.8±42.4 | -24.2±33.4 | -30.3±26.9 | -44.4±43.2 | -29.2±25.2 |
| Positive Responses: | | | | | |
| - $CO_2$ | 3 (19%) | 13 (18%) | 10 (77%) | 6 (86%) | 12 (75%) |
| - Air | 0 (0%) | 3 (19%) | 3 (23%) | 2 (29%) | 1 (6%) |
| Induced-panic attacks: | | | | | |
| - $CO_2$ | 1 (6%) | 9 (56%) | 7 (54%) | 3 (42.8%) | 11 (69%) |
| - Air | 0 (0%) | 0 (0%) | 1 (8%) | 0 (0%) | 0 (0%) |

STAI-1 scores and Δ% VAS-A are expressed as means ± SD

SP: social phobia, SPA: sporadic unexpected panic attacks, PD: panic disorder, CT: healthy controls

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# Chapter 3

## The 35% $CO_2$ hyperreactivity after one-week of treatment with citalopram and reboxetine in patients with Panic Disorder.

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## Abstract

The effects of short treatments (7 days) with citalopram and reboxetine on the reactivity to inhalations of 35% Carbon Dioxide ($CO_2$)/ 65% Oxygen ($O_2$) were compared in 30 patients with Panic Disorder (PD) who had positive responses to 35% $CO_2$ inhalations. An open study design was applied. The 35% $CO_2$ challenge was performed on days 0 (before starting the treatment) and 7. Anxiety reactivity to $CO_2$ decreased significantly with both drugs but the decrease was significantly stronger in the group treated with citalopram. The rate of patients whose response to $CO_2$ became negative after 7 days was significantly higher in the group treated with citalopram than in the one treated with reboxetine. These results support the idea that the modulation of the serotonergic system is more important for $CO_2$ hyperreactivity than the modulation of the noradrenergic one.

## Introduction

The serotonergic and noradrenergic systems are thought to be involved in the pathogenetic mechanisms of Panic Disorder (PD) and the anti-panic mechanisms of psychotropic medications (Gorman et al 2000a; Gorman et al 2000b; Johnson et al 1995; Sullivan et al 1999). The anti-panic efficacy of imipramine might be mainly due to its serotonergic action (Mavissakalian and Perel 1989, 1996) and the efficacy of selective serotonergic agent in the treatment of PD supports the idea of a central role of the modulation of the serotonergic system in the treatment of panic disorder (Kent et al 1998; Perna et al 2001), even if a role of the noradrenergic system is not excluded as suggested by the influence of fluoxetine, a Selective Serotonergic Re-uptake Inhibitor (SSRIs), on noradrenergic function (Coplan et al 1997). Desipramine, a specific noradrenergic uptake inhibitor, has been shown to improve significantly panic symptomatology (Kalus et al 1991; Lydiard et al 1993) and reboxetine, a selective noradrenaline reuptake inhibitor, sems to be effective in the treatment of panic disorder (Brown, 1999; Bertani et al in press).

Carbon dioxide ($CO_2$) hyperreactivity is considered a biological markers of PD, possibly representing the expression of an abnormal suffocation alarm monitor (Klein 1993) or of an abnormal fear network (Gorman et al 2000a). A reduction of the $CO_2$ hyperreactivity might be considered an expression of a normalization of the pathogenetic mechanisms underlying PD (Bellodi and Perna 1998). Drugs effective in the treatment of PD reduce $CO_2$ hyperreactivity wherease those ineffective do not. Four / six weeks of treatment with SSRIs or imipramine and clomipramine decrease significantly $CO_2$ hyperreactivity in patients with PD (Bertani et al 1995; Bocola et al 1998; Perna et al 2002; Pols et al 1996). The decrease of $O_2$ reactivity with SSRIs and anti-panic TCAs is detectable already after one week of treatment (Bertani et al 1997; Bertani et al 2001; Perna et al 1997). Similar results were found for clonazepam (Beckett et al 1986; Nardi et al 2000). On the contrary, premedications with yohimbine and propranolol as well as a 2 week treatment with buspirone did not reduce significantly $CO_2$ reactivity (Pols et al 1989; Pols et al 1994; Van den Hout 1984). These studies support the idea that $CO_2$ inhalation might be an useful tool for screening antipanic properties of psychotropic drugs.
Our recent study shows that a short treatment with paroxetine is more effective in reducing $CO_2$ hyperreactivity than a reboxetine one, suggesting the idea that the modulation of the serotonergic system might play a more important role than the modulation of the noradrenergic one in the anti-panic drug properties (Perna et al 2004). In order to test this idea, we compared the effect of short treatments (7 days) with citalopram, the most selective serotonergic agent (Hyttel 1994), and with reboxetine, on the reactivity to the 35% $CO_2$ challenge.

## Methods

### Subjects

Thirty patients with PD, with/without Agoraphobia, without other concurrent Axis I disorders except for specific phobias, were recruited over seven months at the Anxiety Disorders Clinical and Research Unit of the Department of Neuropsychiatric Sciences, S. Raffaele Hospital, Milan. Criteria for inclusion was a positive response to the 35% $CO_2$ challenge and a negative response to air-placebo. Reactivity to 35% $CO_2$ was considered a positive response for an increase of anxiety of at least of 26% after the inhalation, according to the threshold obtained by Receiver Operating Characteristic (ROC) curve analyses reported to effectively

separate healthy controls from patients with PD (Battaglia and Perna, 1995). Fifteen patients took citalopram and 15 took reboxetine. Most part of patients of citalopram treatment group had been included in our previous study whose results are reported elsewhere (Bertani et al, 2001). An open study design was applied.

Two senior psychiatrists who assessed patients independently by clinical interview and the MINI International Neuropsychiatric Interview – Plus established consensus diagnoses according to DSM IV criteria (Sheehan et al 1994). Physical examination and medical history were performed to exclude any subjects with significant cardio-circulatory and respiratory diseases, personal and familial history of cerebral aneurysm, significant hypertension (systolic > 180 mm Hg, diastolic > 100 mm Hg), pregnancy or epilepsy. Each patient gave their informed consent after receiving a detailed explanation of the entire procedure carried out in accordance with the Declaration of Helsinki, 1964 and its amendments (Tokyo 1975, Venice 1983 and Hong Kong 1989).

### Drug Treatment

Because previous studies showed an increase of anxiety during the first days of treatment with TCAs and SSRIs in PD, especially if the initial doses are too high or raised too fast (Den Boer and Westenberg 1990; Humble and Wistedt 1992), we used low drug doses. Patients took citalopram 10 mg/day and reboxetine 2 mg/day for the entire week. Since no direct comparison between citalopram and reboxetine in humans is available in literature, we used the same dosages used in our previous studies that pairs ¼ of the mean dosage used in depression (Bertani et al, 2001; Perna et al 2004). No concomitant psychotropic drugs or psychotherapeutic interventions were allowed until the end of the study. None of the patients were on chronic medical treatments and they were also asked to avoid any kind of medication at least for the 2 days preceding the study. None underwent cognitive behavioral intervention programs before and during the study. Six patients (20%) had never taken psychotropic medications, 16 (53%) received low doses of benzodiazepines and 8 (27%) received both benzodiazepines and antidepressants. None had ever received citalopram or reboxetine. Clinical assessment of the severity of panic-phobic symptoms was performed by experienced psychiatrists at days 0 (before the beginning of the trial) and 7 using the Panic Associated Symptoms Scale (PASS) (Argyle et al 1991) to assess frequency of panic attacks, level of anticipatory anxiety, and

phobic avoidance. These yielded a global score (PASS-tot) and subscores for panic attacks (PASS-PA), anticipatory anxiety (PASS-AA) and phobic avoidance (PASS-AV).

### 35% $CO_2$ Challenge

On day 0, before starting treatment, and on day 7, patients inhaled one vital capacity of air-placebo and 35% $CO_2$ - 65% $O_2$, in the afternoon, in random order, at an interval of 30 minutes. At the time of the first challenge, all subjects had to have been off all psychotropic medication for at least 2 weeks. Patients were asked to refrain from alcohol for at least 36 hours, beverages containing xanthines for at least 8 hours and food or smoking for at least 2 hours preceding each test. None of the subjects reported nicotine or caffeine abuse or excessive use (equivalent of 4 or more cups of Italian coffee and of 15 or more cigarettes a day). Female patients were tested including the two sessions (0-7) either in the luteal or in the follicular phase, never in the pre-menstrual phase.

### Apparatus

Two different gas mixtures were used: compressed air (placebo) and a mixture of 35% $CO_2$/ 65% $O_2$. Both gases were inhaled through the same self-administration mask. Vital capacity was measured by a respirometer (Wright Respirometer Mark 20; Ferraris Medical Limited) connected to the self-administration mask. The same respirometer measured the gas volume delivered in each inhalation.

### Procedure

The 35% $CO_2$ test was performed in a double blind, random cross-over design, the entire procedure of the 35% $CO_2$ challenge has been described elsewhere (Perna et al 1995). Briefly, subjects were informed that they would be inhaling two harmless gas mixtures containing different percentages of $CO_2$ and $O_2$, and that they might experience some discomfort that would range from a few neurovegetative symptoms to a definite sensation of anxiety/ discomfort with several somatic and/or cognitive sensations. The word "panic attack" was not mentioned to avoid any negative cognitive bias related to expectation. The brevity (few seconds-one minute) of the panic reaction induced by 35% $CO_2$ inhalation, which completely disappears in few minutes, justifies the latter decision ethically. Vital capacity was measured.

Then each subject inhaled one vital capacity of compressed air or of 35% $CO_2$-65% $O_2$ and at the end of each inhalation, subjects were asked to hold their breaths for 4 seconds. The test was considered valid only if the subject had inhaled at least 80% of the previously measured vital capacity. Immediately before and after each inhalation (Air or $CO_2$), anxiety was evaluated on a Visual Analogue Scale for anxiety (VAS-A) describing the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable), a valid scale for the evaluation of the reaction (Battaglia and Perna 1995)
A panic symptoms list was administered to patients before and after the inhalation to calculate the number of symptoms increased after the challenges. The Human Ethics Committee of San Raffaele Hospital approved the 35% $CO_2$ test procedure.

### Data Analysis

The reactivity to the 35% $CO_2$ challenge was assessed both quantitatively and qualitatively.
(I) Quantitatively, to avoid baseline influence on the quantitative assessment of 35% $CO_2$ reactivity, the reactivity was evaluated as Δ% VAS-A (the percentage of maximum increment or decrement possible on the VAS-A scale) (Perna et al 1994).
Δ% VAS-A was calculated as follows:

a. if Δ VAS-A (post-$CO_2$ VAS-A values minus pre-$CO_2$ VAS-A values) was positive, then Δ% VAS-A = Δ VAS-A x 100/(100- VAS-A before $CO_2$).
b. if Δ VAS-A was negative, than Δ% VAS-A = Δ VAS-A x 100/VAS-A before $CO_2$.

(II) Qualitatively, the rate of negative responders (Δ% VAS-A<26) and of patients with at least a 50% decrease of Δ% VAS-A after one week of treatment.
Since previous studies showed no significant effect of the order of gases administration (Battaglia and Perna 1995; Gorman et al 1990), we have not included this variable in the analyses.

### Baseline anxiety

Baseline anxiety was assessed with VAS-A before $CO_2$ scores. We evaluated the modification of baseline anxiety during the first week of treatment using a Multivariate Analysis of Variance (MANOVA) in which Time (T) (Day 0 and day 7) was the repeated measures factor, Drug (D) was the grouping factor and VAS-A before $CO_2$ inhalation scores was the dependent variable.

### 35% $CO_2$ provoked anxiety

We evaluated the changes of Δ% VAS-A in the citalopram and reboxetine groups separately using Wilcoxon test. We compared the modification of 35% $CO_2$-provoked anxiety in the two groups using a Multivariate Analysis of Variance (MANOVA) in which Time (T) (Day 0 and day 7) was the repeated measures factor, Drug (D) was the grouping factor and Δ% VAS-A was the dependent variable.

Mc Nemar's test for paired comparisons was applied to compare the rate of positive responses before and after drug treatment. Differences in the rates of patients with at least a 50% decrease of Δ% VAS-A or with negative responses after one week of treatment in citalopram and reboxetine groups were compared by Fisher'exact test.

### Clinical symptomatology

The effect of one-week treatment with citalopram and reboxetine on the severity of panic-phobic symptomatology was assessed by MANOVAs in which Time (T) (Day 0 and day 7) was the repeated measures factor, Drug (D) was the grouping factor and PASS-tot, PASS-PA, PASS-AA or PASS-AV were the dependent variable. Spearman's test was applied to evaluate the relationship between PASS scores and VAS-A post-$CO_2$ or Δ% VAS-A scores on days 0 and 7.

## Results

### Baseline assessment

There were no significant differences between citalopram and reboxetine groups for age, age at onset of PD, educational level and PASS scores before treatment, presence of agoraphobia and sex distribution (table 1). No differences were found for VAS-A before $CO_2$, VAS-A after $CO_2$, Δ% VAS-A on day 0 (table 2).

At baseline, before the beginning of the treatment, there were no significant linear correlations between Δ% VAS-A and PASS scores.

### Baseline Anxiety

MANOVA did not show any significant effects for VAS-A before $CO_2$ (table 2).

### Clinical symptomatology

MANOVA showed a significant Time effect for PASS-tot ($F=5.3$, $df=1,28$, $p<0.05$). Analysing PASS subscales, a significant decrease of anticipatory anxiety scores ($F=16.8$, $df=1,28$, $p<0.01$) but not of panic attacks and avoidant behavior scores (table 1) was found. Drug effect and Drug x Time interaction were not significant.

### 35% $CO_2$ provoked anxiety

On day 7, the response to the $CO_2$ challenge became negative in a significantly higher rate of patients in the citalopram group (9/15, 60%) than in the reboxetine group (3/15, 20%) (Fisher exact test two tailed, $p< 0.05$). McNemar's tests showed a significant decrease of rates of positive responses after 7 days of treatment with citalopram ($p<0.05$) while the decrease was not significant in the reboxetine-treated group. Similarly, the percentage of patients with at least a 50% decrease of $CO_2$ reactivity was significantly higher (Fisher's exact test two tailed: $p<0.01$) in the citalopram-treated group (11/15, 72%) than in the reboxetine-treated one (3/15, 20%).

The Wilcoxon test showed a significant decrease of $\Delta$% VAS-A in the reboxetine ($Z=2.7$; $p<0.01$) and in the citalopram ($Z=3.1$; $p<0.01$) groups separately. MANOVA showed significant Time effects ($F=57.9$; $df=1,28$; $p<0.01$) and DxT interactions ($F=5.7$; $df= 1,28$; $p<0.05$) for $\Delta$% VAS-A. Both drugs induced a significant decrease of $\Delta$% VAS-A after 7 days of treatment but the decrease was stronger in citalopram treated-group (table 2).

## Discussion

Our study shows that a short treatment of both citalopram and reboxetine is able to reduce $CO_2$ hyperreactivity but the effect of citalopram is significantly stronger than that of reboxetine. Patients showed a significant decrease of $\Delta$% VAS-A after one week of both treatments, but the citalopram treated group showed a higher rate of negative responses to $CO_2$ at the end of the trial than the reboxetine treated one. Clinical and demographic characteristics and baseline anxiety levels before the challenges do not explain our results. Our results show a reduction of anticipatory anxiety already after one week of treatment with both drugs. It might be explained or by an early anxiolitic activity of citalopram and reboxetine or by psychoeducational intervention of at the first session. However, the reduction of $CO_2$ reactivity cannot be

explained by the decrease of anticipatory anxiety, since there were no correlations between anticipatory anxiety and both VAS-A post-$CO_2$ and Δ% VAS scores on day 7.
The decrease of $CO_2$ reactivity obtained in the citalopram group is comparable to that reported in our previous studies with SSRIs (Bertani et al 1997; Perna et al 2002), confirming the ability of serotonergic anti-panic agents to precociously decrease $CO_2$ hyperreactivity in more than 50% of patients with PD. On the contrary, although reboxetine induces a significant decrease of $CO_2$ reactivity, it seems to be less powerful in normalizing the $CO_2$ reactivity in most of the patients. Althought the main limit of this study is the open design, the results parallel the findings of our previous study showing a significantly weaker decrease of $CO_2$ reactivity after short treatment with reboxetine than with paroxetine (Perna et al 2004); since citalopram is the most selective serotonergic agent among SSRIs, this study might confirm the idea that the modulation of the serotonergic system might be more important than the modulation of the noradrenergic one in the treatment of PD.
This idea is strongly supported also by clinical studies that showed a stronger efficacy of mainly serotonergic psychotropic drugs than mainly noradrenergic ones. Fluvoxamine showed significant anti-panic properties whereas maprotiline, a specific noradrenergic drug, did not (Den Boer and Westenberg 1988). Clomipramine, a powerful serotonergic re-uptake inhibitor, showed stronger anti-panic properties than both imipramine (Modigh et al 1992) and desipramine, a specific noradrenergic re-uptake inhibitor (Sasson et al 1999). We recently showed that twelve weeks treatment with reboxetine leads to a significantly weaker reduction of panic attacks than with paroxetine, whereas the reduction of avoidance and anticipatory anxiety was similar with both treatments (Bertani et al in press). It suggested a possible different role of the serotonergic and noradrenergic systems in the modulation of mechanisms underlying PD. The serotonergic system might modulate particularly the "core" phenomenon of PD, i.e. the panic attack, whereas the noradrenergic one might be more involved in clinical phenomena usually following the panic attacks, i.e. the anticipatory anxiety and the avoidant behaviors (Bertani et al in press; Bellodi et al 2003).
The anti-panic efficacy of serotonergic drugs might be linked to the influence of the serotonergic system on the respiratory control mechanisms (Bianchi et al 1995; Mueller et al 1982). This idea is supported by studies showing that a decrease in serotonergic tone, because of tryptophan depletion, increases ventilation during room air breathing in patients with PD but

not in healthy subjects (Kent et al 1996) and anxiety reaction after hypercapnic inhalation in healthy subjects and in patients with PD (Klaassen et al 1998; Miller et al 2000; Schruers et al 2000). Alternatively, serotonin reuptake inhibitors might act in the treatment of PD modulating the "fear network" and might attenuate $CO_2$ hyperreactivity by modulating the inputs to the amygdala that, in turn, influences the respiration by the parabrachial nucleus (Hashimoto et al 1996; Stutzmann and LeDoux 1999). There are also evidences linking the noradrenergic system to respiration. $CO_2$ inhalation enhances firing of the noradrenergic locus coeruleus (Elam et al, 1981) and local tissue acidosis in locus coeruleus increases phrenic nerve activity (Coates et al 1993). Exposure to $CO_2$ increases activity in the adrenergic neurons in the rostral ventrolateral medulla suggesting that noradrenergic neurons are part of the neural network involved in respiratory control mechanisms (Rentero et al, 1997).

Main limitations in our study must be noted. (a) The study had an open design. (b) It is not placebo controlled, even if previous studies did not find a significant placebo effect on 35% $CO_2$ reactivity. (c) The weaker ability of reboxetine to reduce $CO_2$ hyperreactivity could be the result of the used low dose and we cannot be sure that the two dosages used, citalopram 10 mg and Reboxetine 2 mg, have a comparable re-uptake inhibitor power. Further studies using higher dosages might clarify this doubt. We cannot also exclude that reboxetine might have similar effects on $CO_2$ reactivity than citalopram but needs more days of treatment. Finally, the idea that the differential effect on $CO_2$ reactivity found in our study might predict differences in clinical utility should be tested in specific studies.

In conclusion, our results suggest that although both citalopram and reboxetine reduce $CO_2$ hyperreactivity, the serotonergic drug have a stronger $CO_2$ induced-anti panic effect. Our results support the idea that the drugs acting on the serotonergic system might be more effective in the treatment of panic disorder than those acting on the noradrenergic one.

Table 1. Demographic and clinical characteristics of the sample.

| | Citalopram (n=15) | Reboxetine (n=15) |
|---|---|---|
| Age (yrs) | 34.1±9.8 | 33.4±7.9 |
| Education level (years) | 13.9±4.1 | 14.2±3.3 |
| Age at onset (years) | 23.8±5.9 | 24.9±7.2 |
| Sex (males) | 7/15 | 5/15 |
| Agoraphobia | 9/15 | 10/15 |
| PASS: | | |
| - Global score: | | |
| day 0 | 9.5±4.2 | 9.2±4.6 |
| day 7 | 6.9±3.3 | 5.8±3.2 |
| - PASS-PA | | |
| day 0 | 3.9±2.7 | 4.1±3.6 |
| day 7 | 3.5±2.9 | 3.7±2.0 |
| - PASS-AA | | |
| day 0 | 3.9±1.8 | 4.2±1.7 |
| day 7 | 2.1±1.5 | 2.2±1.3 |
| - PASS-AV | | |
| day 0 | 1.1±0.9 | 0.9±0.6 |
| day 7 | 0.8±1.0 | 0.7±0.8 |

Values are expressed as mean ± SD and number.
PAAS, Panic Associated Symptoms Scale; PASS-PA, subscores for panic attacks; PASS-AA, subscores for anticipatory anxiety; PASS-AV, subscores for phobic avoidance.

Table 2. $CO_2$ induced-anxiety across one week of treatment with Citalopram and Reboxetine.

| | Citalopram (n=15) | Reboxetine (n=15) |
|---|---|---|
| Day 0: | | |
| - VAS-A before $CO_2$ inhalation | 35.1±24.3 | 30.1±25.2 |
| - VAS-A after $CO_2$ inhalation | 84.6±12.6 | 82.3±15.8 |
| - Δ% VAS-A | 76.5±16.0 | 72.1±24.9 |
| Day 7: | | |
| - VAS-A before $CO_2$ inhalation | 32.9±21.1 | 24.2±17.4 |
| - VAS-A after $CO_2$ inhalation | 49.7±23.8 | 57.4±22.8 |
| - Δ% VAS-A | 26.0±27.1 | 44.9±23.2 |

Values are expressed as mean ± SD.
VAS-A, Visual Analogue Scale for Anxiety.

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# Chapter 4

## Approximate Entropy of respiratory patterns in Panic Disorder

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## Abstract

**Objective:** Considerable evidence suggests a connection between Panic Disorder (PD) and respiration, but the nature of the respiratory abnormalities in PD remains unclear. We investigated the breath by breath complexity of respiration dynamics in PD.

**Method:** Respiratory physiology assessment was carried out in 40 patients with PD and 31 healthy subjects by using a breath by breath Quarkb2 stationary testing system. Breathing pattern irregularity was measured by applying the Approximate Entropy Index (ApEn).

**Results:** Patients with PD showed significantly higher ApEn indices than healthy controls for the measured respiratory parameters. Sighs contribute to the irregularity of breathing patterns but do not account for all the ApEn differences between patients with PD and healthy controls. Anxiety state, severity of illness, somatic and individual variables such as practicing sports and cigarette smoking did not seem to influence the results.

**Conclusions:** Patients with PD showed higher entropy in respiratory baseline patterns, indicating higher levels of irregularity and complexity in their respiratory function. Higher respiratory entropy could represent a vulnerability factor to panic attacks.

## Introduction

Clinical and experimental evidences suggest a possible role of the respiratory system in the pathophysiology of Panic Disorder (PD). This has led to the idea that a disorderly respiratory control mechanisms may underlie the occurrence of panic attacks (1, 2). Despite the evidence for a connection between panic attacks and respiration, the nature of respiratory abnormalities remains unclear. Some studies found higher respiratory frequency, tidal volume, minute ventilation and lower baseline end-tidal C02 in patients with PD than in patients with other anxiety disorders and healthy controls (3-5), whereas other studies did not confirm these results (6, 7). More univocal and specific respiratory abnormalities arose when the breathing patterns of the patients with PD were studied. Patients with PD showed greater irregularity in tidal volume

than patients with Generalized Anxiety Disorder (8) and greater irregularity in tidal volume and minute ventilation and an increased rate of breathing pauses than healthy subjects (9-12). The irregularity in breathing pattern has been attributed to frequent sighs (8, 10, 11, 13).
In order to clarify the nature of respiratory abnormalities in PD, we investigated the baseline respiratory function of a sample of patients with PD and healthy controls. Since biological systems are characterised by dynamic processes with wide interactions between multiple inputs, non-linear methods are considered the gold standard to measure the complexity of physiological functions (14). Each physiologic signal, such as respiratory or cardiac, usually represents the output of complex mechanisms, including multiple feedback/coupling interactions and inputs from internal and external sources. The moment or linear statistics, such as mean and SD, are unable to analyse the dynamics of complex physiologic signals whereas non-linear statistics unravel highly significant differences in settings in which the former do not distinguish between groups. Many studies stressed the higher value of the non-linear measures, including Fractal Dimension, Correlation Dimension (CD), Largest Lyapunov Exponent (LLE) and Approximate Entropy (ApEn), than linear measures to study the heart rate variability (HRV) (15-18). We used the Approximate Entropy index (ApEn), a non-linear measure of irregularity, to study the dynamics of baseline breathing patterns in our sample. ApEn has been widely applied in endocrine studies (19-21), heart rate studies (22-24) and respiratory physiology studies (25). To date, only three studies have analyzed data from psychiatric populations using the ApEn index (26-28).
The aim of the study was to unravel possible differences in the breath by breath complexity of respiration dynamics in patients with PD and healthy subjects. The contribution of sighing to the irregularity of breathing patterns was assessed. The possible effect of variables that might influence breathing patterns was also evaluated.

## Methods

### Subjects

Forty outpatients with PD with/without Agoraphobia (21 women and 19 men) and 31 healthy subjects (16 women and 15 men) were recruited among those consecutively referred to the Anxiety Disorders Clinical and Research Unit of San Raffaele Hospital, Milan, over a period of 8 months. The healthy controls were recruited by advertisements placed around the University.

Psychiatric diagnosis was obtained by the MINI International Neuropsychiatric Interview for DSM IV-Plus (29). Healthy subjects were free of lifetime psychiatric disorders. Concurrent psychiatric disorders, except specific phobias, were exclusion criteria for patients with PD. The severity of clinical symptomatology in patients with PD was measured by the Panic Associated Symptoms Scale (PASS), which assesses panic attacks (PASS-PA subscale), anticipatory anxiety (PASS-AA subscale) and agoraphobia (PASS-AGO subscale) (30) and the Fear Questionnaire (FQ) which assesses agoraphobia, blood-injury phobia and social phobia (31).
Exclusion criteria for all subjects were significant concurrent cardio-circulatory and respiratory diseases, significant hypertension (systolic > 180 mm Hg, diastolic > 100 mm Hg), pregnancy or epilepsy, according to a direct physical examination and a collection of medical histories. The number of subjects regularly practising sports, the hours of sports activity per week and the number of smokers were recorded.
Respiratory physiology was assessed using the Quarkb2 system.
Before respiratory assessment, subjects had to have been off all psychotropic medications for at least 2 weeks before the tests. None of the patients took fluoxetine in the 6 months before. Because many substances can affect respiratory patterns (32), subjects were asked to refrain from alcohol for at least 36 hours, from beverages or food containing xanthines for at least 8 hours, from non-steroid anti-inflammatory drugs for at least 36 hours and from any food or smoking for at least 2 hours before respiratory physiology assessment.
All participants gave their written informed consent to the study after a detailed explanation of the entire procedure.

### Testing of Respiratory Physiology

Respiration dynamics were assessed by using the Quark b2 stationary testing system (Cosmed, Rome, Italy), which allows assessment of respiration physiology by monitoring respiratory function and pulmonary gas exchange on a breath by breath basis. The breath by breath recording by the Quark b2 system is widely used in sports medicine and respiration physiology studies, in accordance with the recommendations of the American Thoracic Society and the European Respiratory Society (33, 34).

## Apparatus

The Quark b2 system consists of a mobile unit containing the principal components connected on-line to a computer to allow continuous breath by breath recording of respiratory parameters. The principal components are a) a digital infrared light turbine measuring respiration air flows, b) rapid response oxygen ($O_2$) and carbon dioxide ($CO_2$) analyzers c) electronic sensors measuring barometric pressure, ambient temperature and humidity d) a humidity absorber. Before each test, the turbine and the analyzers were calibrated in order to maintain optimal technical characteristics of the apparatus. An open light face mask connects the subject to the respiratory testing system.

## Procedure

Recording of respiratory parameters was carried out by medical doctors trained in the use of the Quark b2 system. A standardized procedure was used throughout to minimize any confounding influences (32). The recording was carried out in a quiet room and took 20 minutes. Patients were recorded between 4 p.m. and 6 p.m. to avoid biases related to circadian rhythms of respiratory control (35, 36). Before the recording started, subjects rested for 20 minutes and were familiarized with the study apparatus.

The subjects were told that the Quarkb2 system assesses baseline respiratory physiology and records the respiratory parameters during natural breathing of resting subjects. The subjects were instructed to remain seated silently, quietly and with eyes open during the entire session. They were also told they could stop the session whenever they wanted with a hand signal to the examiner. Before the start of the recording, baseline anxiety was assessed by the State Trait Anxiety Inventory (37) for state anxiety. A Visual Analogue Scale for anxiety (VAS-A), which describes the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable), was administered immediately before, after 10 minutes from the beginning and at the end of the session.

During the whole procedure, the examiner monitored on a computer screen the continuous recording of respiratory parameters breath by breath and interacted with the subjects only at standardized time intervals to administer the psychometric scales. Any disturbances like coughs, sneezes or laughs that could modify the respiratory pattern were noted by the examiner directly on the data file during the continuous recording, without interrupting the test.

### Assessment of respiratory physiology

Respiratory physiology was assessed by the following parameters: respiratory rate (RR), tidal volume (TV), minute ventilation (MV), ratio between tidal volume and time in inspiration (TV/IT), end-tidal partial pressure of $CO_2$ ($PetCO_2$), ratio between minute ventilation and end-tidal partial pressure of $CO_2$ ($MV/PetCO_2$), ratio between minute ventilation and end-tidal partial pressure of $O_2$ ($MV/PetO_2$). TV/IT, $MV/PetCO_2$ and $MV/PetO_2$ are believed to reflect the Central Nervous System inspiratory drive and chemosensitivity to $CO_2$ and $O_2$, respectively (32, 38). For each respiratory parameter we calculated the mean, the average within-subject standard deviations (SDs), an indicator of the variability of the measure, and the approximate entropy index, an indicator of the irregularity and the "disorder" of the measure (14). The first 3 minutes of recording were discarded in order to minimize any possible influence that familiarization with face mask and study apparatus could have on the respiratory pattern. Likewise, distortions during the breath by breath recording due to artifacts, like coughs, sneezes or laughs, were discarded.

### Assessment of sighs

We assessed the number of sighs in breathing patterns. We defined a sigh as any breath that was at least 500 ml larger than the mean of the prior three breaths and at least 400 ml larger than the following breath, according to the definition used by Abelson and coworkers (11). When there were three or more successive such breaths they were considered a hyperventilatory run and not counted as a sigh (11).

### Statistical Analysis

### Approximate Entropy Index (ApEn)

To quantify the irregularity of each time series, we used the Approximate Entropy (ApEn) index, a model-independent statistic whose mathematical properties and biological applications have been described elsewhere (14, 22). Briefly, the ApEn index is a nonnegative number assigned to a time series, with larger values corresponding to greater apparent process irregularity and smaller values corresponding to more instances of recognizable patterns in the data. Two input parameters, m and r, must be specified to compute ApEn: m measures the

"length" of a sequence of contiguous observations (a run), and r measures the amount of noise in the data that is filtered out in the ensuing calculation. ApEn measures the likelihood that runs that are close (within r) for m observations remain close (within the same tolerance width r) when m is incremented. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and, conversely, random data (high irregularity) produces higher ApEn values. In this study, we calculated ApEn (m, r) values for all data sets using m=1 and r= 20% of the SD of the individual subject's time series. Normalizing r to each time series SD gives ApEn a translation- and scale-invariance. Computational aspects have been described in great detail by Pincus (39). Previous studies that included both theoretical analysis and clinical applications (23, 40) have demonstrated that these input parameters produce good statistical validity (reproducibility) for ApEn applied to the time-series of the lengths considered here. We analyzed 17 minutes of recording with sampling rate=1, sample every 5 seconds and a typical sequence of data of approximately 200 data points.

### Average within-subject standard deviation (SDs)

To quantify the overall variability of each measured parameter, we used the average within-subject standard deviation (SDs). SDs measures the magnitude of the deviation from the mean value for each parameter in each subject.

In summary, SDs and ApEn values quantify two different characteristics of time-series data and provide complementary information. SDs describes the overall variability of a parameter over a period of time, whereas ApEn describes the dynamic pattern of that variability. For instance, tracings of a physiologic parameter with similar overall variability (SDs) might have a regular pattern over time (low ApEn), indicating low complexity of the system, or, on the contrary, an irregular pattern (high ApEn), indicating higher complexity of the system (41).

### Data analyses

Parametric statistical analyses were employed. The continuous data were analyzed by t-test, Analysis of Variance (ANOVA) and Multivariate Analysis of Covariance (MANCOVA). The nominal data were compared by chi-square analysis. The correlation between variables was tested using Pearson Correlation Coefficient.

## Results

In the PD patient group, illness duration was 7.1 ± 7.9 years. Twenty-eight (70%) patients were agoraphobic. PASS total score was 7.0 ± 4.1 and PASS-PA, PASS-AA, PASS-AGO subscales scores were 3.5 ± 2.5, 2.3 ± 2.0, 0.6 ± 0.9, respectively. FQ total score was 45.7 ± 24.5 and FQ-AGO, FQ-BI, FQ-SOC subscales scores were 14.7 ± 12.8, 18.8 ± 12.0, 12.2 ± 8.1, respectively.

There were no significant differences for gender distribution, age, weight, height, BMI, number of subjects who regularly practised sports and hours of sports activity per week between the two groups, whereas there were significantly more smokers among PD patients than healthy controls (Table1).

ANOVA showed that patients with PD had significantly higher baseline anxiety levels before respiratory physiology assessment, as measured by STAI-I scores (45.6 ± 11.6), than healthy controls (29.2 ± 4.1), ($F= 56.8$, $df = 1, 69$, $p<0.01$). VAS-A scores pre-Respiratory Assessment (RA), during-RA and post-RA in patients with PD and in healthy controls were respectively 36.5±25.3, 31.7±27.1, 22.9±24.9 and 7.0±8.7, 4.1±5.6, 2.7±4.7. ANOVA for repeated measures showed significant Diagnosis (D) ($F= 43.0$, $df= 1, 68$, $p<0.01$) and Time (T) ($F= 8.1$, $df= 2, 138$, $p<0.01$) effects for VAS-A scores, while no significant effect of TxD interaction was found.

### Baseline anxiety

MANCOVA with STAI as covariate showed no significant differences for the mean values of all the respiratory parameters between patients with PD and healthy controls (RR=16.53±4.04 and 16.33±3.72, respectively; TV=0.55±0.20 and 0.51±0.17, respectively; MV=8.53±2.98 and 7.87±2.13, respectively; TV/IT=0.38±0.13 and 0.34±0.10, respectively; $PetCO_2$=32.43±4.60 and 32,89±3.07, respectively; $MV/PetCO_2$=0.27±0.16 and 0.24±0.07, respectively; $MV/PetO_2$=0.08±0.03 and 0.07±0.02, respectively), whereas significantly higher average within-subject standard deviations (SDs) were observed in patients with PD than in healthy controls ($R=3.2$; $df=7, 62$, $p<0.01$). Post-hoc Duncan comparisons showed significantly higher SDs in patients with PD for all respiratory parameters except for $MV/PetCO_2$ (Table 2).

MANCOVA with STAI as covariate showed significantly higher ApEn indices of baseline respi-

ratory parameters in patients with PD than in healthy controls (R= 2.6, df= 7, 62, $p<0.02$). Post-hoc Duncan comparisons showed significantly higher ApEn indices in patients with PD than in healthy controls for all respiratory parameters (Table 3).
Similar results were obtained including VAS-A pre respiratory assessment score as covariate in MANCOVA (data available on request).

### Gender

MANCOVA with STAI as covariate and Diagnosis and Gender (G) as grouping factors showed significant Gender effects for mean values of respiratory parameters (R=6.0, df=7, 60, $p<0.01$), whereas no significant Diagnosis effect or DxG interaction was found. Male subjects showed significantly higher mean values of TV, MV, TV/TI, $PetCO_2$, MV/PetO2 than female subjects (Post-hoc Duncan comparisons, $p<0.01$ for all parameters), whereas mean values of RR and $MV/PetCO_2$ were not significantly different (data available on request). MANCOVA with STAI as covariate and Diagnosis and Gender as grouping factors showed a Diagnosis effect for SDs (R=3.0, df=7,60, $p<0.01$) and ApEn indices (R=2.5, df=7,60, $p<0.05$) but did not show either significant Gender effect or DxG interaction for SDs and ApEn indices of all respiratory parameters.

### Smoking

MANCOVA with STAI as covariate and Diagnosis and Smoking (S) as grouping factors showed a significant Diagnosis effect for SDs (R=2.5, df=7, 60, $p<0.05$) and ApEn indices (R=2.2, df=7, 60, $p<0.05$) but not for mean values of respiratory parameters. No significant Smoking effects for mean values, SDs and ApEn indices of all respiratory parameters were found. No significant DxS interaction was found for mean values and ApEn indices for all respiratory parameters, whereas a significant effect was found for SDs (R=2.5, df=7, 60, $p<0.05$). Post-hoc Duncan comparisons showed higher SDs in smoker patients with PD than in smoker healthy controls for RR (3.1±1.6 and 1.6±0.5, respectively, $p<0.01$), TV (0.2±1.1 and 0.1±0.1, respectively, $p<0,05$), MV (2.3±1.4 and 1.2±0.4, respectively, $p<0.01$), $PetCO_2$ (1.9±0.9 and 1.2±0.4, respectively, $p<0.01$), $MV/PetO_2$ (0.03±0.03 and 0.01±0.003, respectively, $p<0.05$), TV/Ti (0.1±0.1 and 0.05±0.017, respectively, $p<0.01$).

### Sports

MANCOVA with STAI as covariate and Diagnosis and Sports (SP) as grouping factors showed a significant Diagnosis effect for SDs ($R=3.4$, $df=7, 60$, $p<0.05$) and ApEn indices ($R=2.3$, $df=7, 60$, $p<0.05$) but not for mean values of respiratory parameters. No significant Sports effect or DxSP interaction for mean values, SDs and ApEn indices of all respiratory parameters were found.

### Clinical characteristics

Linear Pearson Correlation did not show any significant correlation between ApEn indices of all respiratory parameters and illness duration or severity of clinical symptomatology, measured by the PASS and FQ global and subscale scores.

### Sighs

T-test showed higher number of sighs in breathing patterns of patients with PD than in healthy controls ($4.6\pm6.8$ and 0, respectively, $t=3.8$, $df=69$, $p<0.01$). Twenty-one (52.5%) patients with PD showed sighs whereas 19 (47.5%) did not. None of healthy controls showed sighs in their breathing patterns. There were no significant differences for gender distribution, age, weight, height, BMI, number of subjects who regularly practised sports, hours of sports activity per week, illness duration or severity of clinical symptomatology between patients with sighs and without sighs. We compared mean values, SDs and ApEn indices of all respiratory parameters in patients with sights, patients without sighs and healthy controls. MANCOVA with STAI as covariate showed no significant differences for the mean values of all the respiratory parameters in the three groups. MANCOVA with STAI as covariate showed significant Group effect for SDs ($R=6.68$, $df=14, 122$, $p<0.01$). Post-hoc Duncan comparisons showed higher SDs in patients with sighs than in healthy controls for all respiratory parameters ($p<0.01$), higher SDs in patients with sighs than in patients without sighs for RR, TV, MV, $PetCO_2$ ($p<0.01$), whereas showed no differences between patients without sighs and healthy controls for all respiratory parameters (data available on request). MANCOVA with STAI as covariate showed significant Group effect for ApEn indices ($R=4.36$ $df=14, 122$, $p<0.01$). Post-hoc Duncan comparisons showed higher ApEn indices in patients with sighs than in healthy controls for all respiratory parameters ($p<0.01$), higher ApEn indices in patients with sighs than in patients

without sighs for RR (1.62±0.16 and 1.27±0.18, respectively, $p<0.01$), MV (1.59±0.17 and 1.32±0.13, respectively, $p<0.01$), $PetCO_2$ (1.58±0.16 and 1.22±0.31, respectively, $p<0.01$), $MV/PetO_2$ (1.62±0.16 and 1.35±0.11, respectively, $p<0.01$), $MV/PetCO_2$ (1.55±0.18 and 1.27±0.12, respectively, $p<0.01$), TV/Ti (1.54±0.18 and 1.30±0.13, respectively, $p<0.01$) and higher ApEn indices in patients without sighs than in healthy controls for RR and TV (Table 4). Similar results were obtained defining a sigh as >2.0 times the mean TV, according to the definition used by Wilhelm and coworkers (13) (data available on request).
In patients with sighs, the Linear Pearson Correlation did not show any significant correlation between ApEn indices of all respiratory parameters and number of sighs.
None of tested subjects experienced panic attacks or asked to stop the recording during respiratory physiology assessment.

## Discussion

The main finding of our study is that patients with PD had significantly higher approximate entropy indices (ApEn) and higher average within-subject standard deviations (SDs) than healthy controls for the measured respiratory parameters, whereas mean values of respiratory function did not discriminate between the two groups. Our findings suggest that patients with PD have not only greater overall variability but also higher entropy in respiratory baseline patterns, indicating higher levels of irregularity and complexity in their respiratory functions. This is in agreement with a recent study that found higher values of ApEn and LLE, a measure of chaos, for the lung volumes in patients with PD than in normal subjects (27).
Baseline anxiety was higher in patients than in controls, but was not able to explain our respiratory findings. Moreover, the VAS-A scores during the procedure decreased similarly in both groups, indicating that the procedure was no more anxiogenic for patients than for controls. Severity of illness did not influence respiratory pattern irregularity too. Other variables that could influence respiratory pattern, that are age, weight, height and BMI, did not differ between patients and controls.
Male subjects had higher mean values of some respiratory parameters than female, as expected from the physiological difference in body mass (32), whereas no differences were found for respiratory patterns. Sports activity of the subjects tested did not influence the measured respiratory parameters. According to epidemiological studies, smokers were more

prevalent among patients than controls (42). Smoker patients with PD have a higher variability in some respiratory parameters than smoker controls, however they did not differ on the irregularity of breathing patterns. In brief, main somatic and individual variables related to respiration are unable to explain the difference in respiratory pattern found.

### Approximate Entropy and sighs

Our findings not completely overlap with previous studies that found a greater irregularity of tidal volume due to frequent sighs in patients with PD (8, 10, 11, 13). In our study the patients with PD showed significantly higher number of sighs than healthy controls but the presence of sighs does not fully explain the irregularity in breathing patterns. Although the group of patients with sighs showed higher ApEn indices for the respiratory parameters, except for TV, than the group of patients without sighs, the latter showed significantly higher ApEn indices for RR and TV than healthy controls. These findings suggest that sighs contribute to the irregularity of breathing patterns but do not account for all the ApEn differences between patients with PD and healthy controls. At least for the two main respiratory parameters RR and TV the irregularity of breathing patterns in patients with PD is not attributable to the presence of sighs. Moreover, the lack of correlation between ApEn indices for all the respiratory parameters and the number of sighs support the possibility that sighs do not fully explain the irregularity of pattern also for the other respiratory parameters.

Contrary to ApEn indices, SDs for all the respiratory parameters did not differ between patients without sighs and healthy controls. This finding confirms that a linear measure of variability is unable to fully discriminate between patients and controls for a highly complex function such as the respiration, whereas a non-linear measure does so (Fig. 1).

The role of sighs in patients with PD is unclear. Since the sighs have a physiologic function in maintaining normal lung volumes and reducing unpleasant respiratory sensations (43), they could be a compensatory mechanism to the abnormal respiratory function found in the patients with PD. Alternatively, the sighs could be the expression of a higher severity of abnormal function in the rhythm-generating respiratory network (see below). Further studies are necessary to clarify these issues.

## Implications of Approximate Entropy for pathophysiology of Panic Disorder

Entropy describes the amount of randomness or disorder in processes and systems. Recent theories describe living organisms as highly complex and dynamic structures that display a meta-equilibrium around homeostatic levels, oscillating between order and disorder (44, 45). External/internal perturbations can lead a biological system to a state with a high degree of instability, defined as a "bifurcation point", from which the system may proceed to diverging states, such as a new level of order or, on the contrary, to a "disruption", such as a pathological phenomenon (46). The higher entropy in the respiratory function of patients with PD may indicate an intrinsic instability state in respiratory homeostasis on which different critical inputs could act as "disrupting" factors leading to panic attacks. Respiratory instability may underlie the susceptibility of patients with PD to hypercapnic challenges (1).

The question of whether higher respiratory entropy could be a consequence of PD or a trait marker of vulnerability to PD is open, however, two recent studies support the latter hypothesis. Coryell and co-workers reported abnormal respiratory patterns in healthy first-degree relatives of patients with PD breathing a 5% $CO_2$ gas mixture (47). Preliminary data from our team showed higher approximate entropy in baseline respiratory patterns in healthy children of patients with panic disorder compared with those of healthy subjects (48). Instability in respiratory systems might lead to the onset of the disease when the system fails to cope with the stimuli and failed to restore the state of equilibrium. Daily tobacco smoking increase the risk for onset of panic attacks and disorder (42). Since nicotine seems to modulate neurotransmission in the respiratory brainstem network (49), an intrinsic respiratory instability might underlie the development of the disorder after the onset of smoking.

The source of higher respiratory entropy in patients with PD is unclear. Respiration is modulated by a complex regulatory system in which the brainstem plays a central role containing the pacemaker respiratory neurons and the neural network that shape respiratory patterns (50). Irregularity in breathing patterns might arise from an abnormal function of the rhythm-generating network, leading to a lack of physiological synchronicity in inspiratory and expiratory neuron activity. Since limbic and cortical areas influence respiration (51, 52), physiologic instability could also originate from brain centers higher than the brainstem. However, the role of higher centers is questioned by the absence of an influence of state anxiety on respiration instability and by the reported lack of influence of cognitive manipulation on doxapram-induced tidal volume irregularity in patients with PD (11).

The higher respiratory irregularity might also influence the heart rate of patients with PD. Several studies demonstrated decreased HRV (53-55) and one study decreased chaos in HR time series in patients with PD (56). Since the cardiac activity is regulated by direct connections between respiratory and sympathetic/vagal cardiac centres within the brainstem, an abnormal function of the respiratory network could affect the autonomic regulation of the cardiac activity leading to an abnormal modulation of the heart rate.
Finally, the higher respiratory entropy in patients with PD does not necessarily imply a specific intrinsic instability in the respiratory system but it might arise from a more global abnormality in the brainstem neuronal circuits regulating physiological homeostasis functions (57). This idea is supported by the observation that patients with PD show subclinical abnormalities in balance system function (58).
In conclusion, this study shows higher levels of irregularity and complexity in respiratory functions of patients with PD. This finding supports the idea of an abnormal regulation of the respiratory system as a key mechanism in PD. Higher respiratory entropy could represent a vulnerability factor to panic attacks. Further studies are necessary to confirm the specificity of the results comparing patients with PD and with other anxiety disorders. The identification of specific brain structures related to this abnormal respiration feature by brain imaging techniques could help to build a much needed neuroanatomical model of "respiratory" panic.

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Table 1. Demographic and epidemiological characteristics of the sample

| | Patients with PD (n=40) | Healthy Subjects (n=31) | P |
|---|---|---|---|
| Age | 34.2 (11.6) | 33.0 (8.2) | ns |
| Sex (females) | 21 (52.5%) | 16 (52%) | ns |
| Weight (Kg) | 63.9 (12.1) | 68.9 (11.6) | ns |
| Heigth (m) | 1.69 (0.1) | 1.72 (0.1) | ns |
| BMI | 22.2 (3.0) | 23.1 (2.9) | ns |
| Smokers* | 23 (57.5%) | 9 (29%) | <0.05 |
| Sport activity | | | |
| Subjects regularly practising sports | 14 (35%) | 17 (54.9%) | ns |
| h/week | 1.7 (3.9) | 1.5 (1.9) | ns |

Values are expressed as mean (SD) and number (%);
h/week, hours of sports activity per week.
* $X2=5.72$, df=1

Table 2. Whithin-Subjects Standard deviations of respiratory parameters

| | Patients with PD (n=40) | Healthy controls (n=31) | Post-hoc Duncan Analyses |
|---|---|---|---|
| SDs RR | 2.73 (1.35) | 2.12 (0.8) | p<0.05 |
| SDs TV | 0.19 (0.13) | 0.11 (0.05) | p<0.01 |
| SDs MV | 2.13 (1.40) | 1.30 (0.51) | p<0.01 |
| SDs TV/IT | 0.12 (0.14) | 0.06 (0.02) | p<0.05 |
| SDs $PetCO_2$ | 1.83 (0.90) | 1.07 (0.30) | p<0.01 |
| SDs $MV/PetCO_2$ | 0.08 (0.10) | 0.05 (0.06) | ns |
| SDs $MV/PetO_2$ | 0.02 (0.02) | 0.01 (0.01) | p<0.05 |

Values are expressed as mean (SD)
SDs, average within-subject standard deviations; RR, respiratory rate; TV, tidal volume; MV, minute ventilation; TV/IT, inspiratory drive; $PetCO_2$, end-tidal CO2 partial pressure; $MV/PetCO_2$, index of $CO_2$ chemosensitivity; $MV/PetO_2$, index of $O_2$ chemosensitivity.

Table 3. Approximate Entropy indices* of respiratory parameters

| | Patients with PD (n=40) | Healthy controls (n=31) | Post-hoc Duncan analyses |
|---|---|---|---|
| ARR | 1.44 (0.26) | 1.13 (0.20) | p<0.01 |
| ATV | 1.31 (0.21) | 1.11 (0.19) | p<0.01 |
| AMV | 1.44 (0.22) | 1.29 (0.13) | p<0.01 |
| ATV/IT | 1.41 (0.22) | 1.27 (0.17) | p<0.01 |
| $APetCO_2$ | 1.38 (0.33) | 1.09 (0.20) | p<0.01 |
| $AMV/PetCO_2$ | 1.40 (0.23) | 1.23 (0.16) | p<0.01 |
| $AMV/PetO_2$ | 1.47 (0.22) | 1.31 (0.13) | p<0.01 |

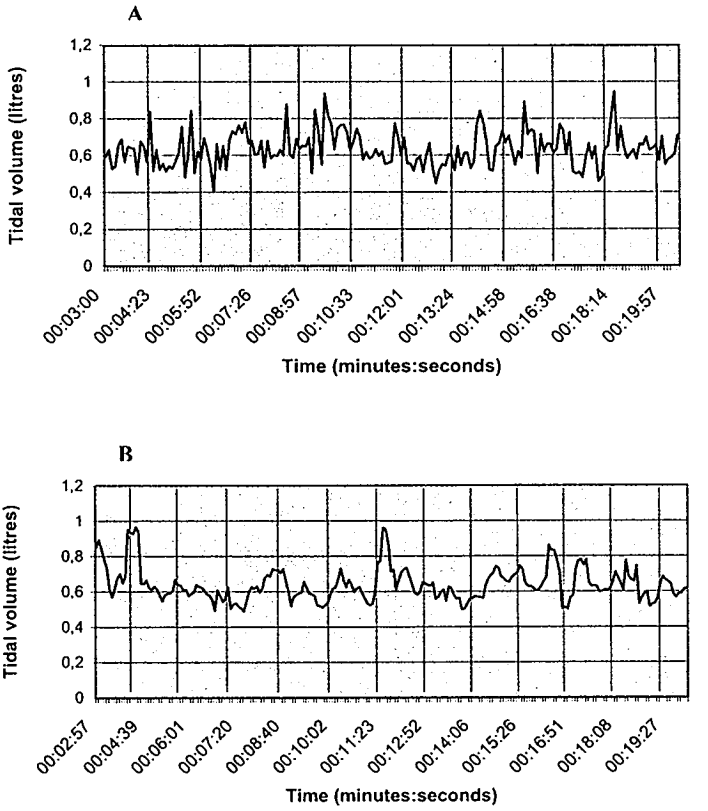
Values are expressed as mean (SD)
ARR, ApEn of respiratory rate; ATV, ApEn of tidal volume; AMV, ApEn of minute ventilation; ATV/IT, ApEn of inspiratory drive; $APetCO_2$, ApEn of end-tidal $CO_2$; $AMV/PetCO_2$, ApEn of $CO_2$ chemosensitivity; $AMV/PetO_2$, ApEn of $O_2$ chemosensitivity.
* Larger values correspond to greater irregularity in the process

Table 4. Approximate Entropy indices* of respiratory parameters

| | Patients with PD without sighs (n=19) | Healthy controls (n=31) | Post-hoc Duncan analyses |
|---|---|---|---|
| ARR | 1.27 (0.18) | 1.13 (0.20) | p<0.01 |
| ATV | 1.37 (0.21) | 1.11 (0.19) | p<0.01 |
| AMV | 1.32 (0.13) | 1.29 (0.13) | ns |
| ATV/IT | 1.30 (0.13) | 1.27 (0.17) | ns |
| $APetCO_2$ | 1.22 (0.31) | 1.09 (0.20) | ns |
| $AMV/PetCO_2$ | 1.27 (0.12) | 1.23 (0.16) | ns |
| $AMV/PetO_2$ | 1.35 (0.11) | 1.31 (0.13) | ns |

Values are expressed as mean (SD)
ARR, ApEn of respiratory rate; ATV, ApEn of tidal volume; AMV, ApEn of minute ventilation; ATV/IT, ApEn of inspiratory drive; $APetCO_2$, ApEn of end-tidal $CO_2$; $AMV/PetCO_2$, ApEn of $CO_2$ chemosensitivity; $AMV/PetO_2$, ApEn of $O_2$ chemosensitivity.
* Larger values correspond to greater irregularity in the process

Figure 1. Breathing patterns with similar overall variability (whithin-subjects Standard Deviation, SDs) and significantly different Approximate Entropy (ApEn) indices* in a patient with Panic Disorder who did not sigh during testing and a healthy comparison subject




A: Patient without sigh; SDs=0.09, ApEn=1.65

B: Healthy control; SDs=0.09, ApEn=1.21

* Larger values correspond to grater apparent irregularity in the process

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# Chapter 5

## 35% $CO_2$ induced panic and respiratory function in Panic Disorder

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## Abstract

Background
It remains unclear if the behavioural hyperreactivity to $CO_2$ in patients with Panic Disorder (PD) is related to an abnormal respiratory function.
Aims
To investigate if higher baseline respiratory irregularity might explain the behavioural hyperreactivity to hypercapnic stimulation in PD.
Method
We examined the relationship between the behavioural reactivity to 35% $CO_2$ inhalation and the baseline respiratory patterns in a sample of patients with PD. The Approximate Entropy (ApEn) index, a non-linear measure of irregularity, was applied.
Results
The patients who panicked during $CO_2$ inhalation have higher baseline respiratory irregularity than patients who did not panic. The higher irregularity of tidal volume is a respiratory predictor of panic response to $CO_2$ inhalation.
Conclusions
The behavioural hyperreactivity to $CO_2$ might be related to a higher irregularity and complexity of baseline respiratory patterns, thus supporting the idea of an abnormal respiratory function in PD.

## Introduction

Carbon dioxide ($CO_2$) inhalation induces panic-like attacks in patients with PD (Griez, de Loof, Pols, et al, 1990; Perna, Battaglia, Garberi, et al, 1994). It is unclear if the behavioural reactivity to $CO_2$ is related to an abnormal respiratory function. Klein linked $CO_2$ hyperreactivity to an altered suffocation alarm monitor, representing a primary respiratory abnormality in PD

(Klein, 1993), whereas Gorman and coworkers proposed a central role of an abnormal fear network (Gorman, Kent, Sullivan, et al, 2000).
Patients with PD have higher irregularity in their respiratory baseline patterns and chaotic breathing in comparison with healthy subjects (Caldirola, Bellodi, Caumo, et al, 2004; Yeragani, Radhakrishna, Tancer, et al, 2002a). In this study we investigated the relationship between 35% $CO_2$ behavioural reactivity and baseline respiratory patterns in a sample of patients with PD. Our hypothesis was that higher irregularity in respiratory patterns might explain the behavioural reactivity to hypercapnic stimulation.

## Methods

### Subjects

Fifty-seven outpatients with PD with/without Agoraphobia (30 women and 27 men) and 31 healthy subjects (16 females and 15 males) were included in the study. The former group was recruited among those consecutively referred to the Anxiety Disorders Clinical and Research Unit of San Raffaele Hospital, Milan, over a period of 12 months. The latter group was recruited by advertisements placed around the University. Both the whole healthy subjects group and most part of the outpatients are the same included in a previous study with different aims and whose results are reported elsewhere (Caldirola, Bellodi, Cammino, et al, 2004 submitted).
Psychiatric diagnosis was obtained by the MINI International Neuropsychiatric Interview-Plus for DSM IV (Sheehan, Lecrubier, Janavs, et al, 1994). Healthy subjects were free from lifetime psychiatric disorders. Concurrent psychiatric disorders, except specific phobias, were exclusion criteria for patients with PD. The severity of clinical symptomatology in patients with PD was measured by the Panic Associated Symptoms Scale (PASS), which assesses panic attacks (PASS-PA subscale), anticipatory anxiety (PASS-AA subscale) and agoraphobia (PASS-AGO subscale) (Argyle, Deltito, Allerup, et al, 1991) and the Fear Questionnaire (FQ) which assesses agoraphobia, blood-injury phobia and social phobia (Marks & Mathews, 1979).
Exclusion criteria for all subjects were significant concurrent cardio-circulatory and respiratory diseases, significant hypertension (systolic > 180 mm Hg, diastolic > 100 mm Hg), pregnancy or epilepsy, according to a direct physical examination and medical history.

## Procedure

Each patient underwent 1) the respiratory physiology assessment by the Quark b2 stationary testing system and, after an interval of 30 minutes, 2) the 35% $CO_2$ challenge test. The healthy subjects underwent the respiratory physiology assessment only.

Subjects had to have been off all psychotropic medications for at least 2 weeks before the tests. None of the patients took fluoxetine in the 6 months before. Since many substances could affect respiratory patterns (Akiyama & Kawakami, 1999), the subjects were asked to refrain from alcohol for at least 36 hours, from beverages or food containing xanthines for at least 8 hours, from non-steroid anti-inflammatory drugs for at least 36 hours and from any food or smoking for at least 2 hours before starting the procedure.

All participants gave their written informed consent to the study after a detailed explanation of the entire procedure. The Human Ethics Committee of San Raffaele Hospital approved the protocol.

## Quark b2 stationary testing system

The Quark b2 stationary testing system (Cosmed, Rome, Italy) allows assessment of respiration physiology by monitoring respiratory function on a breath by breath basis, in accordance with the recommendations of the American Thoracic Society and the European Respiratory Society (Palange, Forte, Onorati, et al, 2000; Schena & Padoin, 1999).

The apparatus and the entire procedure has been described in details elsewhere (Caldirola, Bellodi, Cammino, et al, 2004 submitted; Caldirola, Bellodi, Caumo,et al, 2004). Briefly, the Quark b2 system consists of a mobile unit containing the principal components connected on-line to a computer to allow continuous breath by breath recording of respiratory parameters. An open light face mask connects the subject to the respiratory testing system. A standardized procedure was used throughout to minimize any confounding influences (Akiyama & Kawakami, 1999). The recording was carried out in a quiet room and took 20 minutes. Patients were recorded between 4 p.m. and 6 p.m. to avoid biases related to circadian rhythms of respiratory control (Spengler, Czeisler & Shea, 2000). Before the recording started, subjects rested for 20 minutes and were familiarized with the study apparatus. The subjects were told that the Quarkb2 system assesses baseline respiratory physiology and records the respiratory parameters during natural breathing of resting subjects. The subjects

were instructed to remain seated silently, quietly and with eyes open during the entire session. They were also told they could stop the session whenever they wanted with a hand signal to the examiner. Before the start of the recording, baseline anxiety was assessed by the State Trait Anxiety Inventory (Spielberg, Gorsuch & Lushere, 1970) for state anxiety. A Visual Analogue Scale for anxiety (VAS-A), which describes the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable), was administered immediately before, after 10 minutes from the beginning and at the end of the session.
During the whole procedure, the examiner monitored on a computer screen the continuous recording of respiratory parameters breath by breath and interacted with the subjects only at standardized time intervals to administer the psychometric scales. Any disturbances like coughs, sneezes or laughs that could modify the respiratory pattern were noted by the examiner directly on the data file during the continuous recording, without interrupting the test.

### Assessment of respiratory physiology

Respiratory physiology was assessed by measuring the Respiratory Rate (RR), and the Tidal Volume (TV). For each respiratory parameter we calculated the mean, the average within-subject standard deviations (SDs), an indicator of the variability of the measure, and the Approximate Entropy index, an indicator of the irregularity and the "chaos" of the measure (Pincus, 1991). The first 3 minutes of recording were discarded in order to minimize any possible influence that familiarization with face mask and study apparatus could have on the respiratory pattern. Likewise, distortions during the breath by breath recording due to artifacts, like coughs, sneezes or laughs, were discarded.

### 35% $CO_2$ challenge test

Two different gas mixtures were used: compressed air (placebo) and a mixture of 35% $CO_2$/ 65% $O_2$. Patients inhaled one vital capacity of air-placebo and 35% $CO_2$ - 65% $O_2$, in random order, at an interval of 30 minutes. Both gases were inhaled through the same self-administration mask. Vital capacity was measured by a respirometer (Wright Respirometer Mark 20; Ferraris Medical Limited) connected to the self-administration mask. The same respirometer measured the gas volume delivered in each inhalation.
The 35% $CO_2$ challenge test was performed in a double blind, random cross-over design.

The entire procedure has been described elsewhere (Perna, Battaglia, Garberi, et al, 1994). Briefly, subjects were informed that they would be inhaling two harmless gas mixtures containing different percentages of $CO_2$ and $O_2$, and that they might experience some discomfort that would range from a few neurovegetative symptoms to a definite sensation of anxiety/discomfort with several somatic and/or cognitive sensations. The word "panic attack" was not mentioned to avoid any negative cognitive bias related to expectation. The brevity (few seconds-one minute) of the panic reaction induced by 35% $CO_2$ inhalation, which completely disappears in few minutes, justifies the latter decision ethically. Vital capacity was measured. Then each subject inhaled one vital capacity of compressed air or of 35% $CO_2$-65% $O_2$ and at the end of each inhalation, subjects were asked to hold their breaths for 4 seconds. The test was considered valid only if the subject had inhaled at least 80% of the previously measured vital capacity. Before the 35% $CO_2$ challenge, baseline anxiety was assessed by the State Trait Anxiety Inventory for state anxiety (Spielberg, Gorsuch & Lushere, 1970). The responses to the challenges were evaluated by a Visual Analogue Scale for Anxiety (VAS-A) and the Panic Symptom List (PSL) performed by the subjects immediately before and after each inhalation (Air or $CO_2$). Visual Analogue Scale for anxiety (VAS-A) describes the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable) and is considered a valid scale for the evaluation of the reaction (Battaglia & Perna, 1995); PSL is a self-rating questionnaire assessing the 13 panic symptoms described in DSM-IV on a five-point scale (0=absent, 1=mild, 2=moderate, 3=severe, 4=very intense) (Pols, Zandbergen, de Loof, et al, 1991). The Human Ethics Committee of San Raffaele Hospital approved the 35% $CO_2$ test procedure.

### Assessment of 35% $CO_2$ reactivity

The global anxiety reactivity to the 35% $CO_2$ challenge was evaluated as Δ% VAS-A (the percentage of maximum increment or decrement possible on the VAS-A scale), to avoid baseline influence on the quantitative assessment of 35% $CO_2$ reactivity (Perna, Battaglia, Garberi, et al, 1994). Δ% VAS-A was calculated as follows. If Δ VAS-A (post-$CO_2$ VAS-A values minus pre-$CO_2$ VAS-A values) was positive, then Δ% VAS-A = Δ VAS-A x 100/(100- VAS-A before $CO_2$). If Δ VAS-A was negative, than Δ% VAS-A = Δ VAS-A x 100/VAS-A before $CO_2$.
From the PSL was obtained the Total Symptom Score (TSS=sum of all the 13 panic symptom scores)

According to the scales described and DSM-IV criteria, the reaction to the 35% $CO_2$ challenge was considered to be an induced panic attack when it included (1) a sensation of fear or panic with at least Δ% VAS-A>26%, an ideal threshold previously shown by a receiver operating characteristic (ROC) analysis (Battaglia & Perna, 1995), and (2) an increase of the scores of at least four symptoms on the PSL.

## Statistical Analysis

### Approximate Entropy Index (ApEn)

To quantify the irregularity of each time series, we used the Approximate Entropy (ApEn) index, a model-independent statistic whose mathematical properties and biological applications have been described elsewhere (Pincus, 1991; Pincus, Gladstone & Ehrenkranz, 1991). Briefly, the ApEn index is a nonnegative number assigned to a time series, with larger values corresponding to greater apparent process irregularity and smaller values corresponding to more instances of recognizable patterns in the data. Two input parameters, m and r, must be specified to compute ApEn: m measures the "length" of a sequence of contiguous observations (a run), and r measures the amount of noise in the data that is filtered out in the ensuing calculation. ApEn measures the likelihood that runs that are close (within r) for m observations remain close (within the same tolerance width r) when m is incremented. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and, conversely, random data (high irregularity) produces higher ApEn values. In this study, we calculated ApEn (m, r) values for all data sets using m=1 and r= 20% of the SD of the individual subject's time series. Normalizing r to each time series SD gives ApEn a translation- and scale-invariance. Computational aspects have been described in great detail by Pincus (Pincus, 2000). Previous studies that included both theoretical analysis and clinical applications (Pincus, Cummins & Haddad, 1993; Pincus, Gevers, Robinson, et al, 1996) have demonstrated that these input parameters produce good statistical validity (reproducibility) for ApEn applied to the time-series of the lengths considered here. We analyzed 17 minutes of recording with sampling rate=1, sample every 5 seconds and a typical sequence of data of approximately 200 data points.

ApEn index has been widely applied in endocrine studies (Roelfsema, Biermasz & Veldhuis, 2002; van den Berg, Pincus, Veldhuis, et al, 1997), heart rate studies (Pincus, Cummins & Haddad, 1993; Pincus, Gladstone & Ehrenkranz, 1991; Pincus & Viscarello, 1992) and respiratory physio-

logy studies (Engoren, 1998). Recently, it has been applied for analyzing data from psychiatric populations too (Caldirola, Bellodi, Caumo, et al, in press; Kaloupek, McWilliams & Keane, 2000; Yeragani, Nadella, Hinze, et al, 2000; Yeragani, Radhakrishna, Tancer, et al, 2002b).

### Average within-subject standard deviation (SDs)

To quantify the overall variability of each measured parameter, we used the average within-subject standard deviation (SDs). SDs measures the magnitude of the deviation from the mean value for each parameter in each subject.

In summary, SDs and ApEn values quantify two different characteristics of time-series data and provide complementary information. SDs describes the overall variability of a parameter over a period of time, whereas ApEn describes the dynamic pattern of that variability. For instance, tracings of a physiologic parameter with similar overall variability (SDs) might have a regular pattern over time (low ApEn), indicating low complexity of the system, or, on the contrary, an irregular pattern (high ApEn), indicating higher complexity of the system (Pincus, 1994).

### Data analyses

Parametric statistical analyses were employed. The continuous data were analyzed by Analysis of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA) and Covariance (MANCOVA). The correlation between variables was tested using Pearson Correlation Coefficient.

In order to identify predictors of panic response to 35% $CO_2$ challenge test, multiple regression analyses were performed by using 9 preselected baseline measures (STAI pre-$CO_2$, VAS-A pre-$CO_2$ and TSS pre-$CO_2$ scores, mean and SDs values and ApEn indices of RR and TV) with induced panic attack as the outcome measure.

## Results

In the group of patients, age, age of onset of PD and illness duration were 35.2 (11.4), 29.1 (9.8) and 6.4 (7.5) years, respectively. Mean age of healthy subjects was 33.0 (8.2). There were no significant differences for gender distribution, age, weight, height, and BMI between the two groups (data not shown).

Thirty-nine (68%) patients were agoraphobic. PASS total score was 7.0 (3.9) and PASS-PA, PASS-AA, PASS-AGO subscales scores were 3.4 (2.4), 2.7 (1.9), 0.8 (0.9), respectively. FQ

total score was 42.6 (25.0) and FQ-AGO, FQ-BI, FQ-SOC subscales scores were 13.8 (13.0), 18.7 (11.7), 12.0 (8.1), respectively.
Patients with PD had significantly higher STAI-I scores before respiratory physiology assessment, (45.6 (11.8)) than healthy subjects (29.2 (4.1)), ($F= 56.9$, $fd = 1, 86$, $p<0.01$). VAS-A scores pre-Respiratory Assessment (RA), during-RA and post-RA in patients with PD and in healthy subjects were respectively 34.3 (25.4), 29.3 (26.5), 23.1 (23.2) and 7.0 (8.7), 4.1 (5.6), 2.7 (4.7). ANOVA for repeated measures showed significant Diagnosis (D) ($F= 36.1$, $fd= 1, 86$, $p<0.01$) and Time (T) ($F= 8.6$, $df= 2, 172$, $p<0.01$) effects for VAS-A scores, while no significant effect of TxD interaction was found.

### Respiratory physiology

MANCOVA with STAI scores as covariates showed higher ApEn indices of baseline respiratory parameters in patients with PD than healthy subjects ($R=6.8$, $fd= 2, 84$, $p<0.01$), whereas no significant differences for the mean values and the average within-subject standard deviations (SDs) of the respiratory parameters between the two groups were found (data not shown). Post-hoc Duncan comparisons showed significantly higher ApEn indices in patients with PD than in healthy controls for both Respiratory Rate (RR), and Tidal Volume (TV) ($p<0.01$) (ApEn RR=1.36 (0.3) and 1.13 (0.2), respectively; ApEn TV=1.28 (0.2) and 1.11 (0.2), respectively). Similar results were obtained including VAS-A pre-RA scores as covariates in MANCOVA (data not shown).

### $CO_2$ reactivity

Thirty-five (61.4%) patients had a $CO_2$-induced panic attack whereas 22 (38.6%) did not. Patients who panicked and those who did not, did not significantly differ for gender distribution, weight, height, Body Mass Index, age, age of onset, illness duration, severity of clinical symptomatology, measured by the PASS and FQ global and subscale scores (data not shown), VAS-A pre-$CO_2$ scores, STAI pre-$CO_2$ scores and TSS pre-$CO_2$ scores (VAS-A pre-$CO_2$= 29.8 (22.8) and 31.9(27.3), STAI pre-$CO_2$= 45.6 (10.7) and 47.6 (14.7), TSS pre-$CO_2$= 4.8 (4.9) and 3.6 (4.7) respectively). Patients who panicked did not significantly differ for baseline anxiety levels before respiratory physiology assessment, as measured by STAI-I scores, from those who did not panic (STA-I= 45.1 (10.8) and 48.1 (13.5), respectively). VAS-A scores

pre-RA, during-RA and post-RA in patients who panicked and in those who did not were respectively 33.8 (26.1), 26.2 (25.6),
21.5 (23.1) and 35.8 (25.3), 34.8 (27.9), 25.6 (23.7). ANOVA for repeated measures showed significant Time effect ($F= 7.8$, $fd= 2, 110$, $p<0.01$) for VAS-A scores, while no significant effects of $CO_2$ reactivity and Time x $CO_2$ reactivity interaction were found.
MANCOVA with STAI scores as covariates showed significant differences for the ApEn indices of baseline respiratory parameters among the patients who panicked, those who did not and the healthy controls ($R= 6.1$, $fd= 4, 166$, $p<0.01$). Post-hoc Duncan comparisons showed that the patients who panicked had higher ApEn indices of Respiratory Rate (RR) and Tidal Volume (TV) than both patients who did not panic and healthy controls ($p<0.01$), whereas the latter two groups did not differ for ApEn indices (Table 1).
No significant differences for the mean and SDs values of the baseline respiratory parameters among the three groups were found (Table 1).
Linear Pearson Correlation did not show any significant correlation between ApEn indices of respiratory parameters and STAI pre-$CO_2$ scores, VAS-A pre-$CO_2$ scores, TSS pre-$CO_2$ scores, STAI-I scores pre-RA, VAS-A scores pre-RA, during-RA and post-RA.
Multiple regression analyses ($R^2$ =0.30, $fd=9,47$, $p<0.05$) identified the ApEn index of Tidal Volume (TV) as baseline predictor of induced panic attacks ( =0.38, $p<0.05$), whereas STAI pre-$CO_2$ scores, VAS-A pre-$CO_2$ scores, TSS pre-$CO_2$ scores, mean and SDs values of RR and TV and ApEn index of RR were not.

## Discussion

Our study showed that patients with PD have higher baseline irregularity in their respiratory patterns than healthy controls, extending our previous results (Caldirola, Bellodi, Cammino, et al, 2004 submitted; Caldirola, Bellodi, Caumo, et al, 2004; Yeragani, Radhakrishna, Tancer, et al, 2002a). We found that (a) the patients who panicked have higher Approximate Entropy indices of the respiratory parameters than both patients who did not panic and healthy subjects, whereas the last two groups did not differ; (b) the higher ApEn index of tidal volume is a respiratory predictor of panic response to $CO_2$ inhalation. On the contrary, mean and SDs values of the respiratory parameters did not differ among patients who panicked, those who did not and healthy controls, and they did not predict the response to $CO_2$ as well as anxiety or symptom levels before the inhalation.

### Respiratory irregularity and induced panic

Our findings suggest that behavioural hyperreactivity to $CO_2$ might be related to a higher irregularity and complexity of baseline respiratory patterns, thus supporting the idea of an abnormal respiratory function in PD. Our results were not explained by individual variables, severity of clinical symptomatology, baseline and procedural anxiety levels during respiratory physiology assessment, and were not correlated with anxiety or symptoms levels before the 35% $CO_2$ challenge test.

Our results are in agreement with previous studies which showed that patients who panicked during $CO_2$ inhalation have greater baseline respiratory variability than patients who did not panic (Bystritsky, Craske, Maidenberg, et al, 2000; Martinez, Kent, Coplan, et al, 2001; Papp, Martinez, Klein, et al, 1997). Several studies have suggested the idea of a "respiratory subtype" of PD associated with an abnormal respiratory function (Biber & Alkin, 1999; Perna, Bertani, Caldirola, et al, 1996) and our results suggest that chaotic breathing might be the expression of that abnormal function. Baseline chaotic breathing might affect the ability of patients to carry out adequate respiratory responses when external or internal changes occur. Since $CO_2$ inhalation stimulates respiration and induces ventilatory response to restore the respiratory homeostasis, the baseline respiratory irregularity might constrain efficient compensatory mechanisms during $CO_2$ challenge test, possibly influencing the occurrence of the induced panic attacks. The physiological ventilatory response to $CO_2$ inhalation mainly involves an increase of tidal volume with a lesser effect on respiratory rate (Honda & Tani, 1999), hence the baseline irregularity of tidal volume might be the "critical factor" which impairs the compensatory respiratory response, thus resulting as predictor of $CO_2$ induced panic. The important role of the tidal volume irregularity in PD is also supported by the findings that tidal volume remains unstable in patients with PD during doxapram-induced stimulation both with or without cognitive manipulations (Abelson, Weg, Nesse, et al, 2001).

### The source of the respiratory irregularity

The source of the higher respiratory irregularity is unclear. Several different mechanisms could be involved (Perna, Caldirola & Bellodi, in press). (a) The respiratory irregularity might be the expression of compensatory mechanisms for keeping the $CO_2$ partial pressure under the treshold of the abnormal suffocation alarm system hypothesized by Donald Klein (Klein, 1993).

(b) Since the respiratory brainstem neural network is able to drive multiple different breathing patterns, such as eupnea, sighs and gasps (Lieske, Thoby-Brisson, Telgkamp, et al, 2000), the respiratory irregularity might arise from an intrinsic abnormal activity in the brainstem respiratory pace-makers neurons and/or in the synchronicity of inspiratory and expiratory neurons, rather than to be the expression of an abnormal chemosensitivity per se. (c) An abnormal regulation of the cardiovascular and balance systems in patients with PD was found (Perna, Alpini, Caldirola, et al, 2001; Yeragani, Nadella, Hinze, et al, 2000). Since these systems and the respiratory one are highly interconnected, a more global disfunction in the neuronal circuits that regulate physiological homeostatic functions might explain the respiratory irregularity. (d) Limbic inputs influence respiratory changes during different emotional states (Boiten, Frijda & Wientjes, 1994), hence, the respiratory irregularity could also originate from brain centers higher than the brainstem, although the lack of influence both of anxiety state on respiratory irregularity and cognitive manipulation on doxapram-respiratory stimulation in patients with PD (Abelson, Weg, Nesse, et al, 2001) do not seem support this idea.

Till now these conjectures remain speculative since the available data are not sufficient for testing the different possible mechanisms underlying the respiratory irregularity. Further studies of the respiratory patterns under different experimental manipulations might help to clarify this issue.

In conclusion, our study suggests that the baseline respiratory irregularity might explain $CO_2$-induced panic in patients with PD. It is unclear if the respiratory irregularity could be a trait or state characteristic. The preliminary findings of both higher respiratory irregularity in children of patients with PD than in children of healthy controls (Perna, Ieva, Caldirola, et al, 2002) and higher $CO_2$ induced ventilatory variability in healthy first-degree relatives of patients with PD than relatives of healthy controls and patients with Major Depression (Coryell, Fyer, Pine, et al, 2001) might support the latter idea.

Our results might support the idea that panic is linked to an abnormal function of the respiratory physiology, although several critical issues should be clarified to fully validate this idea. The main ones are the possible influence of the emotional states on the respiratory abnormalities found in patients with PD and the possible specific neuroanatomical circuits involved in the "respiratory panic".

Table 1. Approximate Entropy (ApEn) indices *, mean and SDs values of respiratory parameters

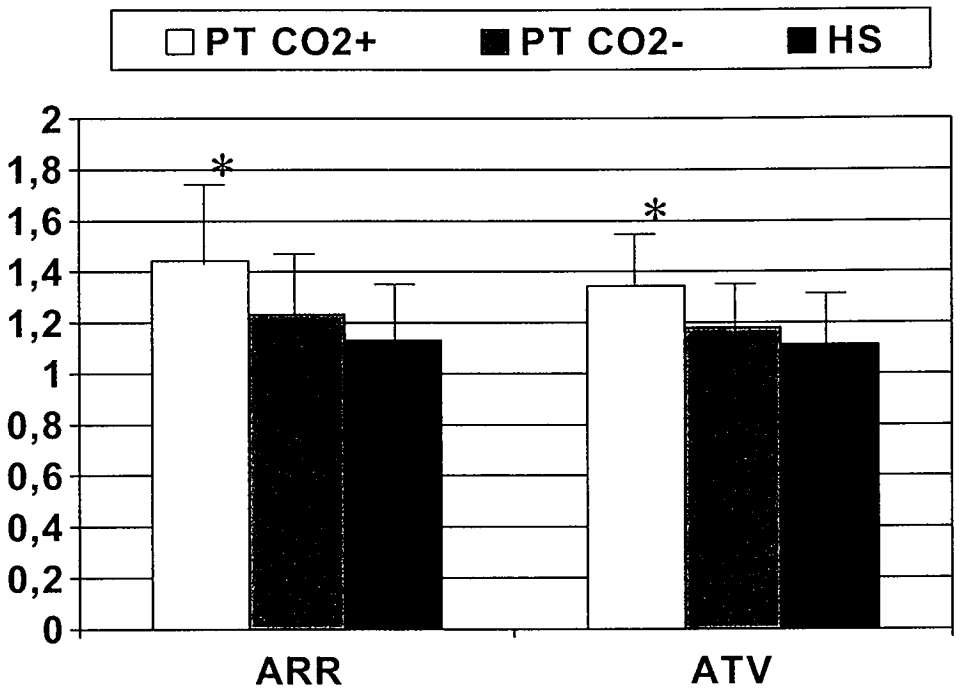
| | Patients who panicked (n=35) | Patients who did not panicked (n=22) | Healthy subjects (n=31) |
|---|---|---|---|
| A RR | 1.44 (0.3) | 1.23 (0.2) | 1.13 (0.2) |
| A TV | 1.34 (0.2) | 1.18 (0.2) | 1.11 (0.2) |
| M RR (breaths/minute) | 16.3 (4.1) | 16.8 (3.8) | 16.3 (3.7) |
| M TV (liters) | 0.5 (0.1) | 0.5 (0.2) | 0.5 (0.2) |
| SDs RR | 2.7 (1.4) | 2.1 (1.0) | 2.1 (0.8) |
| SDs TV | 0.2 (0.1) | 0.1 (0.1) | 0.2 (0.1) |

Values are expressed as mean (SD)

* Larger values correspond to greater irregularity in the process

A, ApEn index; M, mean value; SDs, average within-subject standard deviations; RR, respiratory rate; TV, tidal volume.

Figure 1. Approximate Entropy (ApEn) indices in the three compared groups.




PT $CO_2$+, patients who panicked with $CO_2$ challenge test; PT $CO_2$-, patients who did not panic with $CO_2$ challenge test; HS, Healthy subjects; A, ApEn index; RR, respiratory rate; TV, tidal volume.
*$p<0.01$

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# Chapter 6

## The language of dyspnea in Panic Disorder

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## Abstract

Dyspnea is a key symptom in panic attacks. This study investigated different types of dyspnea induced by the 35% $CO_2$ challenge test given to patients with Panic Disorder (PD). The types of dyspnea provide room for possible conjectures on neurophysiological pathways involved in the experience of breathing discomfort in PD and in the panic-respiration connection. Factor analysis applied to the Dyspnea Questionnaire identified three main factors: Breathing Effort, Sense of Suffocation and Rapid Breath. Factor scores for Breathing Effort and Sense of Suffocation significantly discriminated between patients who did and those who did not report $CO_2$-induced panic attacks. Factor scores for Breathing Effort significantly discriminated between patients whose reaction resembled their unexpected panic attacks and those whose reaction did not. A dissociation between an increased central respiratory command and a decreased mechanical efficiency of the respiratory response in patients with PD may underlie the Breathing Effort factor during the $CO_2$ challenge. The Sense of Suffocation factor was found to be linked to chemosensitivity. Although involved in $CO_2$ reactivity, it may not be a central factor in unexpected panic attacks.

## Introduction

Dyspnea is a common sensation. It can present as a key symptom in certain medical conditions (Meek, 1999) and can also be experienced by healthy subjects (Manning and Mahler, 2001; Simon et al, 1989). Dyspnea is the subjective experience of breathing discomfort that subsumes qualitatively distinct sensations with variable intensity, probably arising from different pathophysiologic mechanisms (Meek, 1999). Since dyspnea and pain share several characteristics, experimental approaches developed in the study of pain have subsequently been applied to investigate dyspnea (Banzett and Moosavi, 2001). Advances in the use of standardized measurement instruments (e.g. pain questionnaires and verbal descriptor scales) have led to the recognition that pain is multidimensional; whence physiology and brain

imaging studies have widely investigated the underlying neural processing of pain sensations (Banzett and Moosavi, 2001). Similarly, dyspnea has been studied in both medical conditions and experimental settings where dyspnea is evoked in healthy subjects in order to focus on the verbal descriptors of dyspnea sensations and the possible mechanisms underlying them. Findings suggest that dyspnea may encompass multiple sensations ill-described by a single term and not explained by a single neurobiological mechanism (Tobin, 1990). Different specific groups of breathing discomfort descriptors have been identified and linked to different possible underlying neurobiological pathways such as the activation of chemoreceptors, pulmonary and/or respiratory muscle receptors and the outgoing respiratory motor command. While numerous factors may influence the subjective perception of dyspnea, verbal descriptors and associated neural pathways are thought to be shared by different conditions (Mahler, 1996; Moy et al, 2000; O'Donnell et al, 1997). Dyspnea is a key symptom in panic attacks. Experimental evidence has demonstrated the role the respiratory system plays in the pathophysiology of Panic Disorder (PD) (Klein, 1993; Bellodi and Perna, 1998; Klein, 2002). Klein emphasized that dyspnea is a salient characteristic of spontaneous panic but not of fear reactions (Klein, 2002). Panicogenic agents, such as sodium lactate and carbon dioxide, provoke panic attacks with prominent dyspnea but without activation of the hypothalamic-pituitary-adrenal axis (HPA), whereas other agents, such as fenfluramine and yohimbine, provoke anxious reactions without marked dyspnea but with activation of the HPA (Hollander et al, 1989; Liebowitz et al, 1985; Shina et al, 1999). These findings support the idea that panic attacks, particularly those characterized by marked dyspnea, are not simply equivalent to fear/stress reactions; they also provide evidence for the "respiratory theory" of the "false suffocation alarm" (Klein, 1993). Despite the existence of a panic-respiration connection (Bellodi and Perna, 1998), its underlying neurophysiological pathways remain unclear. This study investigated types of dyspnea induced by the 35% $CO_2$ challenge test in patients with PD. The types of dyspnea provides room for possible conjectures on the neurophysiological pathways involved in the experience of breathing discomfort in PD and in the panic-respiration connection.

## Methods

### Subjects

Over a period of 9 months, a total of 78 outpatients with PD with/without Agoraphobia (46 women and 32 men; mean age 32.4 ± 8.8 years; mean illness duration 5.6 ± 6.8 years) were recruited among those consecutively referred to the Anxiety Disorders Clinical and Research Unit of San Raffaele Hospital, Milan. Psychiatric diagnosis was obtained by senior psychiatrists using the MINI International Neuropsychiatric Interview for DSM IV-Plus (Sheehan et al, 1994).

Exclusion criteria comprised concurrent psychiatric disorders, except specific phobias, significant concurrent cardiocirculatory and respiratory diseases, personal or family history of cerebral aneurysm, significant hypertension (systolic blood pressure > 180 mm Hg, diastolic blood pressure > 100 mm Hg), pregnancy or epilepsy, according to physical examination and medical history.

The severity of clinical symptomatology in patients with PD was measured using the Panic Associated Symptoms Scale (PASS), which assesses panic attacks (PASS-PA subscale), anticipatory anxiety (PASS-AA subscale) and agoraphobia (PASS-AGO subscale) (Argyle et al, 1991) and the Fear Questionnaire (FQ) which assesses agoraphobia, blood-injury phobia and social phobia (Marks and Mathews, 1979).

### Procedure

Patients were administered the 35% $CO_2$ inhalation challenge test and then asked to fill in the Dyspnea Questionnaire (see below).

Pre-test conditions required that the subjects were not to have taken any medication for at least 2 weeks, and had refrained from alcohol for at least 36 hours, xanthines for at least 8 hours and food or smoking for at least 2 hours before the test. Participants gave their written informed consent after having received a detailed explanation of the test procedure.

### The 35% $CO_2$ inhalation challenge test

Subjects were tested in double-blind random fashion, described in detail elsewhere (Perna et al, 1994). Two different gas mixtures were employed: compressed air (placebo) and a mixture of 35% $CO_2$ and 65% $O_2$. Both gases were inhaled through the same self-administration

mask. Vital capacity was measured by a respirometer (Wright Respirometer Mark 20, Ferraris Medical Limited) connected to the mask. The respirometer measured the gas volume delivered in each inhalation. Subjects were informed that they would be inhaling two harmless gas mixtures containing different percentages of $CO_2$ and $O_2$, and that they might experience some discomfort, ranging from a few neurovegetative symptoms to a definite sensation of anxiety/discomfort with several somatic and/or cognitive sensations. The words "panic attack" were never mentioned. Each subject inhaled one-vital capacity of 35% $CO_2$ and 65% $O_2$ or compressed air in randomly assigned order. After each inhalation, subjects were asked to hold their breath for 4 seconds. An interval of 25-30 minutes elapsed between the two inhalations. The test was considered valid only if the subject had inhaled at least 80% of the previously measured vital capacity.

The responses to the challenges were evaluated using a Visual Analogue Scale for Anxiety (VAS-A) and the Panic Symptom List (PSL) (Pols et al, 1991). The VAS-A describes the degree of global subjective anxiety on a continuum from 0 (no anxiety present) to 100 (worst anxiety imaginable), and the PSL is a self-administered questionnaire assessing each of the 13 symptoms of PD (DSM III-R/IV) on a 5-point scale ranging from 0 (absent) to 4 (very intense) (range, 0-52).

Global anxiety reactivity was evaluated as Δ% VAS-A (percentage of maximum increment or decrement possible on the VAS-A scale) and calculated as follows:

a. if VAS-A (post-$CO_2$ VAS-A values minus pre-$CO_2$ VAS-A values) was positive, then Δ% VAS-A = VAS-A x 100/(100- VAS-A before $CO_2$).

b. if VAS-A was negative, then Δ% VAS-A = VAS-A x 100/VAS-A before $CO_2$.

Before the 35% $CO_2$ challenge, each patient completed the State Trait Anxiety Inventory, state version (STAI) (Spielberger et al, 1970)

## Assessment of $CO_2$ reactivity

### $CO_2$-induced panic attack

According to the scales described above and the DSM IV criteria for panic attack, the reaction to the 35% $CO_2$ challenge was considered to be an induced-panic attack when a sensation of fear or panic with at least Δ% VAS-A ≥ 26 was evoked and there was an increase of the

scores of at least 4 symptoms on the PSL. The VAS-A value is an ideal threshold that a receiver operating characteristic (ROC) analysis of the 35% $CO_2$ challenge has previously been shown able to separate panic patients and healthy controls with a positive predictive power of 91% and a negative predictive power of 75% (Battaglia and Perna, 1995).

### Similarity to unexpected panic attack

In order to identify those patients whose reaction to $CO_2$ resembled their unexpected panic attacks, all subjects were asked to compare their $CO_2$-induced subjective experience with their typical unexpected panic attacks and to rate it as "different from" or "similar to" an unexpected panic attack.

### The Dyspnea Questionnaire

We administered our Italian version of the 19-item Dyspnea Questionnaire developed by Simon and co-workers (Simon et al, 1989) (Table 1). Each item (descriptor) consists of a sentence describing a breathing discomfort sensation. To ensure that the original English version items were accurately translated in our Italian version, four specialists (an Italian English-native language psychiatrist, a psychologist, a pneumologist, a specialist in sport medicine) worked independently on translating the texts. The Italian text was accepted when three of four translators gave identical versions; discrepancies in translation were worked out by consensus among the four translators.

Patients were instructed to choose the items (one or more) they thought most closely described the breathing sensations they experienced during the $CO_2$ challenge test.

### Assessment of dyspnea

Since specific groups of breathing discomfort descriptors are thought to define dyspnea better than a single descriptor (Mahler et al, 1996; Moy et al, 2000; O'Donnell et al, 1997), factor analysis (see below) was applied to the Dyspnea Questionnaire to identify the groups of descriptors (factors) that best defined the type of dyspnea the patients experienced during the $CO_2$ challenge.

Dyspnea experiences were evaluated by sub-grouping patients according to the assessment of $CO_2$ reactivity.

## Statistical Analysis

### Factor Analysis: application in our study

We applied factor analysis to the 19 descriptors (variables) of the Dyspnea Questionnaire. Our sample had more than twice the number of variables subjected to factor analysis, which is the proportion recommended to obtain a good level of reliability. The factor analysis was carried out using principal component extraction in which the axes are not rotated. This method allowed us to extract a few hierarchically ordered factors, wherein the first factor extracted is the most important since it has the highest factor weight. Each factor consists of a group of variables with different factor weights. The higher the factor weight of each variable, the more that variable contributes to the factor. Factor rotation was performed on the data obtained from the previous not rotated factor analysis to obtain variables with very high factor weights in some factors and very low factor weights in others. We chose a varimax rotation which implies orthogonal factors. The maximum number of factors extracted was selected by the Cattel Scree Test. To insert a variable into a factor, a factor weight of at least 0.5 was selected as the threshold value. The factor scores were then computed, which represent a measure of each subject's contribution to each factor.

### Data analyses

Parametric statistical analyses were employed since the data were normally distributed. The continuous data were analyzed by Student's t-test. Wilks lambda discriminant analysis was employed to determine the factor scores that distinguished the sub-grouped patients according to the assessment of $CO_2$ reactivity. The correlation between variables was tested using Pearson Correlation Coefficient. All analyses were made using the Statistica 5.0 software package.

## Results

Of a total of 78 subjects, 67 (85%) patients were agoraphobic; 45 (58%) had a $CO_2$-induced panic attack and 33 (42%) did not; 52 (67%) patients rated the $CO_2$-induced reaction "similar" to their unexpected panic attacks, whereas 26 (33%) did not; 7 (15.5%) of 45 patients with an induced panic attack rated the $CO_2$-induced reaction "different" from their unexpected panic attacks and 14 (42.4%) of 33 without an induced panic attack rated the

$CO_2$-induced reaction "similar" to their unexpected panic attacks (Table 2).
The patient subgroups did not differ significantly in illness duration or in severity of clinical symptomatology as measured by PASS and FQ global and subscale scores, VAS-A pre-$CO_2$ scores and STAI scores (data available on request), except for the higher STAI scores in patients with reactions similar to their panic attacks compared with those whose reactions were different (44.5±10.2 and 38.2±11.4, respectively; $t=2.71$, $df=76$, $p<0.05$). The mean number of chosen descriptors was 5.36±3.97. The frequencies of choice of each descriptor after $CO_2$ inhalation are listed in Figure 1. Descriptor S6, " I cannot take a deep breath", and S1, " My breath does not go in all the way", were chosen by more than 50% of the sample, whereas S16, " I feel that I am breathing more", was chosen by only 5 subjects (6.4%) (Fig. 1). The frequencies of choice did not significantly differ between the genders. The choice of the descriptors was not influenced by age or educational level. Three factors were extracted and rotated by varimax rotation. The descriptors within each factor were ranked starting from those with the highest factor weight to those with the lowest. The names defining each factor were obtained by both interpreting the overall meaning of the descriptors within that factor and by considering that the higher the factor weight, the more each descriptor contributed to the interpretation of that factor. The factors Breathing Effort (F1), Sense of Suffocation (F2) and Rapid Breath (F3) accounted for over 80% of the variance explained (Table 3).
Wilks lambda discriminant analysis showed that the factor scores for the factors Breathing Effort and Sense of Suffocation significantly discriminated between patients reporting $CO_2$-induced panic attacks and those who did not ($F=3.63$, Wilks lambda=0.87, $p<0.01$) and that the factor scores for Breathing Effort significantly discriminated between patients whose reaction resembled their unexpected panic attacks and those whose reaction did not ($F=3.63$, Wilks lambda=0.87, $p<0.01$) (Table 4).
Linear Pearson Correlation showed no significant correlation between STAI scores and factor scores in either the whole sample or the subgroups. The whole sample was also subgrouped using as cut-off values the median scores of the STAI and the VAS-A pre-$CO_2$ scales (42 and 20, respectively). Wilks lambda discriminant analysis showed that the factor scores for F1, F2 and F3 did not significantly discriminate either between patients with a STAI score above and below the median ($n=42$ and $n=35$, respectively) or between patients with a VAS-A pre-$CO_2$ score above and below the median ($n=39$ and $n=39$, respectively) (data available on request).

## Discussion

Our findings show that the three factors of Breathing Effort (F1), Sense of Suffocation (F2) and Rapid Breath (F3) best described the sensations of breathing discomfort induced by the 35% $CO_2$ challenge in the overall sample of patients with PD. The assessment of $CO_2$ reactivity showed that $CO_2$-induced panic attacks and $CO_2$ reactions similar to unexpected panic attacks did not overlap, indicating that the use of different criteria produces different rates of panic (Rassovsky and Kushner, 2003). The discriminating factor scores in patients subgrouped according to the assessment did not completely overlap. The factor scores for Breathing Effort and Sense of Suffocation significantly discriminated between the patients reporting an induced panic attack and those who did not, whereas only the factor scores for Breathing Effort significantly discriminated between the patients whose $CO_2$ reaction resembled their unexpected panic attacks and those whose response did not. Duration of illness, severity of clinical symptomatology and baseline/anticipatory anxiety did not explain the results. Baseline anxiety in patients whose $CO_2$-induced response was similar to their unexpected panic attacks was higher than in those whose response was different; however, it was not correlated with factor scores. Moreover, the factor scores did not significantly discriminate between patients with high or low baseline anxiety and anxiety before the 35% $CO_2$ challenge test.

The dyspnea factors obtained in our sample provide room for possible conjectures on the neurophysiological pathways involved in the experience of breathing discomfort in PD and in the panic-respiration connection. The factor Breathing Effort describes the conscious awareness of muscular effort during activation of respiratory skeletal muscles that is thought to arise from a dissociation between a central respiratory motor command and a mechanical response of the respiratory muscles (Manning and Schwartzstein, 1995; Meek and Schwartzstein, 1999). The association between a panic response to $CO_2$ challenge and the factor Breathing Effort may be explained by the abnormalities found in the respiratory function of patients with PD. A thoracic breathing pattern (Beck and Scott, 1988) and a higher irregularity and instability in baseline breathing patterns are characteristic of patients with PD (Abelson et al, 2001; Caldirola et al, 2004; Wilhelm et al, 2001; Yeragani et al, 2002). These characteristics could affect the ability of patients with PD to maintain an adequate respiratory homeostasis when external or internal changes occur. A dissociation between the increased central respiratory command stimulated by $CO_2$ and a decreased mechanical efficiency of the respiratory response might,

therefore, lead to a heightened sense of Breathing Effort during the $CO_2$ challenge test.
The factor Sense of Suffocation arises mainly from the stimulation of chemoreceptors (Meek and Schwartzstein, 1999) and involves the activation of the limbic/paralimbic and cerebellar regions (Corfield et al, 1995; Banzett et al, 2000; Liotti et al, 2001; Parson et al, 2001; Evans et al, 2002). In our study, the $CO_2$-induced panic attacks were linked to high factor scores for Sense of Suffocation, but this link disappeared after patients were subgrouped according to the similarity of $CO_2$ responses to their unexpected panic attacks. Studies on the chemosensitivity of patients with PD have produced conflicting results; the most recent study showed no abnormal chemoreflex threshold and sensitivity in PD (Katzman et al, 2002). Our findings suggest that while chemosensitivity is involved in $CO_2$ reactivity, it may not be central to unexpected panic attacks. This idea remains speculative, however, since the comparison with unexpected panic attacks was retrospective and indirect. Confirmation of this idea needs further investigation into breathing discomfort after unexpected panic attacks. The Rapid Breath factor, which describes a shallow, rapid breathing pattern in response to an excessive mechanical load (Mahler, 1998; Manning et al, 2001) was not associated with $CO_2$ reactivity.
Besides the role the respiratory control centers play in the regulatio of breathing, the experience of breathing discomfort is probably also modulated by contextual and cognitive influences (Meek and Schwartzstein, 1999). In fact, brain imaging studies have shown that $CO_2$-induced breathlessness in healthy subjects involves the limbic/paralimbic areas (Corfield et al, 1995; Banzett et al, 2000; Liotti et al, 2001); hence, it cannot be excluded that emotional processing could have influenced dyspnea perception in our sample. However, the lack of effect of cognitive behavioral therapy on respiratory hyperactivity to $CO_2$ or of cognitive manipulation on doxapram-induced irregular breathing patterns in patients with PD (Gorman et al, 1997; Abelson et al, 2001) does not support the idea that fear/anxiety fully explains the respiratory discomfort in PD. Recent brain imaging studies have in fact shown that the consciousness of breathlessness activates brain areas like the cerebellum that do not seem to be involved in conditioned fear/anxiety models; instead, such areas probably underlie basic emotions linked to an organism's survival functions (Klein, 2002; Liotti et al, 2001; Parson et al, 2001; Evans et al, 2002). These anatomical circuits may be involved in Klein's "false suffocation alarm" in PD (Klein, 2002). $CO_2$-induced dyspnea may be influenced by an abnormal function in brain circuits processing physiological perceptions/sensations linked to basic homeostatic functions.

Along this line of thought, an abnormal processing of information about the organism's internal milieu in the brain circuits subserving basic emotions (Damasio, 1999) may lead to an abnormal modulation of the respiratory basic emotions in patients with PD.

## Conclusions

In $CO_2$-provoked panic attacks, the sense of Breathing Effort is the most peculiar dyspnea sensation; it may be linked to a decreased efficiency of mechanical responses of the respiratory system in patients with PD, whereas the Sense of Suffocation may be less specific for panic attacks. Further research is needed to explore this idea. For example, imaging studies of specific brain structures related to respiratory sensations may be able to identify the brain circuits possibly involved in "respiratory" panic. Building on such evidence, respiratory training of inspiratory and expiratory muscle function could enhance the overall efficiency of the respiratory system in patients with PD.

Table 1. Dyspnea Questionnaire.

| |
|---|
| S1: My breath does not go in all the way |
| S2: My breathing requires effort |
| S3: I feel that I am smothering |
| S4: I feel a hunger for more air |
| S5: My breathing is heavy |
| S6: I cannot take a deep breath |
| S7: I feel out of breath |
| S8: My chest feels tight |
| S9: My breathing requires more work |
| S10: I feel that I am suffocating |
| S11: I feel that my breath stops |
| S12: I am gasping for breath |
| S13: My chest is constricted |
| S14: I feel that my breathing is rapid |
| S15: My breathing is shallow |
| S16: I feel that I am breathing more |
| S17: I cannot get enough air |
| S18: My breath does not go out all the way |
| S19: My breathing requires more concentration |

Table 2. Assessment of $CO_2$ reactivity.

| | $CO_2$-induced PA (n=45) (a) | No $CO_2$-induced PA (n=33) (b) |
|---|---|---|
| Similarity to unexpected PA (n=52) (c) | 38 | 14 |
| No similarity to unexpected PA (n=26) (d) | 7 | 19 |

Values are expressed as the number of patients who reported a $CO_2$-induced panic attack (a), patients who did not report a $CO_2$-induced panic attack (b), patients whose $CO_2$-induced reaction was similar to their unexpected panic attacks (c), patients whose $CO_2$-induced reaction was different from their unexpected panic attacks (d).

Table 3. Factors obtained by Factor Analysis of the 19 Dyspnea Questionnaire descriptors

| Factors | | Explained variance |
|---|---|---|
| F1: Breathing Effort | S2: My breathing requires effort<br>S17: I cannot get enough air<br>S13: My chest is constricted<br>S11: I feel that my breath stops<br>S1: My breath does not go in all the way<br>S8: My chest feels tight<br>S19: My breathing requires more concentration | 34% |
| F2: Sense of Suffocation | S10: I feel that I am suffocating<br>S12: I am gasping for breath<br>S3: I feel that I am smothering<br>S4: I feel a hunger for more air | 27% |
| F3: Rapid breath | S15: My breathing is shallow<br>S14: I feel that my breathing is rapid<br>S7: I feel out of breath | 20% |

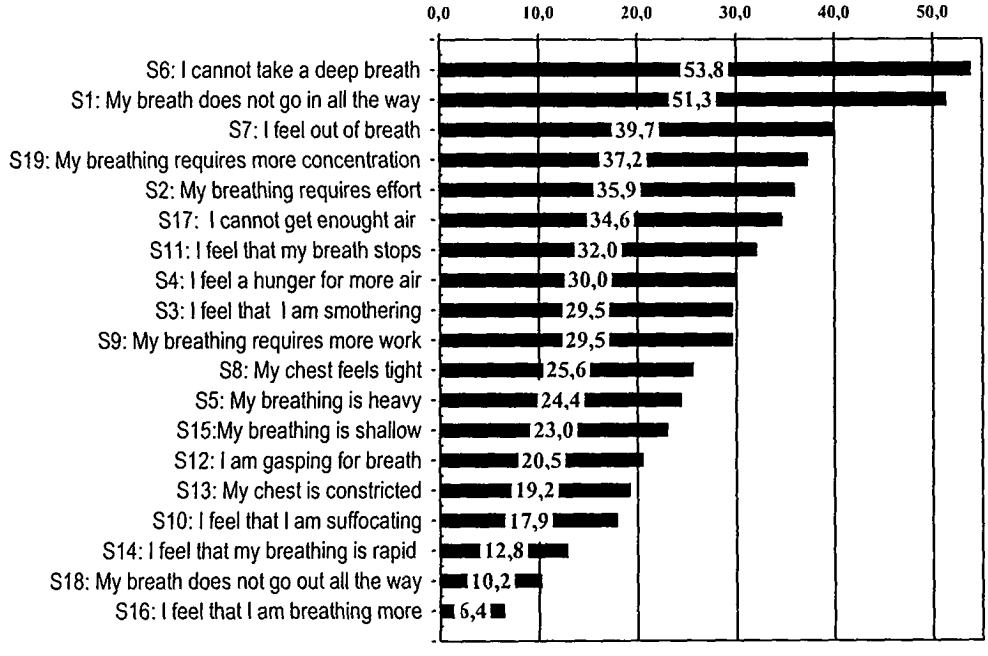
Based on their factor weight, the descriptors (saturation in the factor >0.5) are ordered within each factor from the highest to the lowest value.

Table 4. Discriminant analysis of factor scores in subgroups of patients with PD.

| | Patients with $CO_2$-induced panic attack (n=45) | Patients without $CO_2$-induced panic attack (n=33) | p |
|---|---|---|---|
| F1: Breathing Effort | 0.20 (1.08) | -0.26 (0.81) | p<0.05 |
| F2: Sense of Suffocation | 0.21 (1.05) | -0.30 (0.85) | p<0.05 |
| F3: Rapid Breath | 0.08 (1.15) | -0.12 (0.73) | ns |
| | Patients whose reaction was similar to their unexpected panic attacks (n=52) | Patients whose reaction was different from their unexpected panic attacks (n=26) | |
| F1: Breathing Effort | 0.20 (1.06) | -0.40 (0.72) | p<0.05 |
| F2: Sense of Suffocation | 0.15 (1.01) | -0.30 (0.90) | ns |
| F3: Rapid breath | 0.01 (1.12) | -0.02 (0.71) | ns |

Values are expressed as mean (SD)

Figure 1. Frequency of choice of each descriptor on the Dyspnea Questionnaire



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# Chapter 7

## Smoking and respiratory irregularity in Panic Disorder

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## Abstract

Background
The biological mechanisms underlying the link between smoking and panic attacks are unknown. Smoking might increase the risk of panic by impairing the respiratory system function.
Methods
We evaluated the effect of smoking on the respiratory irregularity in patients with Panic Disorder (PD) and healthy comparison subjects and the role of the respiratory disorders in this effect. We applied the Approximate Entropy index (ApEn), a non-linear measure of irregularity, to study the breath by breath baseline respiratory patterns in our sample.
Results
Both smoker and non-smoker patients have higher irregularity in their respiratory patterns than healthy subjects. Smoker patients showed higher ApEn indices of baseline respiratory rate and tidal volume than non-smoker patients ($R=5.4$, $fd= 2, 55$, $p<0.01$), whereas in healthy subjects smoking does not influence the regularity of the respiratory patterns. The respiratory disorders do not account for the influence of smoking on respiratory irregularity. The smokers have higher severity of the panic attacks than non-smokers.
Conclusions
Smoking might have an impairing influence on vulnerable respiratory function in PD. Smoking might act as a "disrupting" factor specifically on intrinsic baseline respiratory instability of patients with PD, possibly influencing the onset and/or the maintenance of PD.

## Introduction

Epidemiological and clinical studies focusing on anxiety disorders show a strong association between smoking and Panic Disorder (PD) (Amering et al 1999; Breslau and Klein 1999;

Isensee et al 2003; Jacobs et al 1990; Johnson et al 2000; Pohl et al 1992). Daily smoking is associated with an increased risk for later onset of panic attacks or disorder (Amering et al 1999; Breslau and Klein 1999; Isensee et al 2003; Pohl et al 1992), with a higher risk in active than past smokers (Breslau and Klein 1999). The biological mechanisms underlying this association are still unknown. Since PD is characterized by an abnormal respiratory function (Bellodi and Perna 1998; Gorman et al 1988; Klein 1993; Klein 1994; Martinez et al 1996; Papp et al 1993; Papp et al 1997), smoking might increase the risk of panic by impairing the respiratory system function. Breslau and Klein suggested an indirect influence of smoking on panic by increasing the risk of lung diseases that could affect the putative "false suffocation alarm system". However, the higher incidence of PD also in smokers without lung diseases suggested the possible involvement of other different mechanisms (Breslau and Klein 1999). Smoking could act also by a receptor sensitization in the carotid body by the cigarette carbon monoxide (Breslau and Klein 1999). As well, a possible misinterpretation of physical symptoms, induced by nicotine assumption or withdrawal, might trigger panic attacks (Isensee et al 2003).

The aim of this study is to investigate the possible mechanisms linking smoking and respiratory function in PD. Two recent studies, applying non-linear statistics, reported higher entropy in respiratory baseline patterns in patients with PD, indicating higher levels of irregularity and complexity in their respiratory functioning, that might be a possible vulnerability factor for panic attacks (Caldirola et al 2004; Yeragani et al 2002). Therefore, in this study we investigated (1) the possible effect of smoking on the irregularity of the baseline respiratory patterns in a group of patients with PD and healthy comparison subjects; (2) the possible role of the lung diseases in the explanation of this effect; (3) the influence of smoking on the clinical symptomatology.

Since non-linear methods are considered the gold standard to measure the complexity of physiological functions (Pincus 1991), we applied the Approximate Entropy index (ApEn), a non-linear measure of irregularity, to study the breath by breath baseline respiratory patterns in our sample (Caldirola et al 2004; Yeragani et al 2002).

## Methods and Materials

### Subjects

Fifty-eight outpatients with PD with/without agoraphobia (29 females and 29 males) and 31 healthy subjects (16 females and 15 males) were included in the study. They were recruited among those consecutively referred to the Anxiety Disorders Clinical and Research Unit of San Raffaele Hospital, Milan, over a period of 12 months. The healthy controls were recruited by advertisements placed around the University. Both the whole healthy subjects group and 40 patients are the same included in a previous study with different aims and whose results are reported elsewhere (Caldirola et al 2004). Psychiatric diagnosis was obtained by the MINI International Neuropsychiatric Interview-Plus for DSM IV (Sheehan et al 1994). Healthy subjects were free of lifetime psychiatric disorders. Concurrent psychiatric disorders, except specific phobias, were exclusion criteria for patients with PD. The severity of clinical symptomatology in patients with PD was measured by the Panic Associated Symptoms Scale (PASS), which assesses panic attacks (PASS-PA subscale, range of score: 0-17), anticipatory anxiety (PASS-AA subscale, range of score: 0-7) and agoraphobia (PASS-AGO subscale, range of score: 0-3) (Argyle et al 1991) and the Fear Questionnaire (FQ) which assesses agoraphobia (range of score, 0-40), blood-injury phobia (range of score, 0-40) and social phobia (range of score, 0-40) (Marks and Mathews 1979).

Exclusion criteria for all subjects were significant concurrent cardio-circulatory and respiratory diseases, significant hypertension (systolic > 180 mm Hg, diastolic > 100 mm Hg), pregnancy or epilepsy, according to a direct physical examination and medical history. Inclusion criteria on smoking basis are described in details below.

Before respiratory assessment, subjects had to have been off all psychotropic medications for at least 2 weeks before the tests. None of the patients took fluoxetine in the 6 months before. Since many substances could affect respiratory patterns (Akiyama and Kawakami 1999), the subjects were asked to refrain from alcohol for at least 36 hours, from beverages or food containing xanthines for at least 8 hours, from non-steroid anti-inflammatory drugs for at least 36 hours and from any food or smoking for at least 2 hours before respiratory physiology assessment.

### Smoking assessment

In this study we included (1) non smokers, i.e. subjects that declared they had never used cigarettes or other tobacco products in their lifetime and (2) smokers, i.e. subjects with an active tobacco use on daily basis and with a regular smoking habit. Regular smokers were defined as subjects that smoked on daily basis for a period of at least four weeks continually (Breslau and Klein 1999; Isensee et al 2003; Sonntag et al 2000) and did not quit smoking for a period longer than six month continually in their lifetime. Regular smokers were also classified as non dependent-smokers if they had never met DSM-IV criteria for nicotine dependence and as dependent-smokers if they had.

The age of onset of smoking is defined as the age at which the subjects started to smoke daily for at least four weeks (Breslau and Klein 1999; Isensee et al 2003; Sonntag et al 2000). In our sample all the subjects smoked cigarettes except one healthy subjects that smoked cigars. The number of cigarettes/cigars smoked daily were also assessed.

### Respiratory disorders assessment

The respiratory disorders were assessed directly by medical history and by checking the medical reports of each patient. Specifically, the lifetime occurrence, the age of onset and the lifetime course of asthma, asthmatic bronchitis, bronchitis, pneumonia, emphysema and tuberculosis were assessed.

All participants gave their written informed consent to the study after a detailed explanation of the entire procedure. The Human Ethics Committee of San Raffaele Hospital approved the protocol.

### Respiratory assessment

### The Quark b2 stationary testing system

The Quark b2 stationary testing system (Cosmed, Rome, Italy) allows assessment of respiration physiology by monitoring respiratory function on a breath by breath basis, in accordance with the recommendations of the American Thoracic Society and the European Respiratory Society (Palange et al 2000; Schena and Padoin 1999). The apparatus and the entire procedure has been described in details elsewhere (Caldirola et al 2004). Briefly, the Quark b2

system consists of a mobile unit containing the principal components connected on-line to a computer to allow continuous breath by breath recording of respiratory parameters. An open light face mask connects the subject to the respiratory testing system.

### Procedure

A standardized procedure was used throughout to minimize any confounding influences (Akiyama and Kawakami 1999). The recording was carried out in a quiet room and took 20 minutes. Patients were recorded between 4 p.m. and 6 p.m. to avoid biases related to circadian rhythms of respiratory control (Spengler et al 2000; Stephenson et al 2000). Before the recording started, subjects rested for 20 minutes and were familiarized with the study apparatus. The subjects were told that the Quarkb2 system assesses baseline respiratory physiology and records the respiratory parameters during natural breathing of resting subjects. The subjects were instructed to remain seated silently, quietly and with eyes open during the entire session. They were also told they could stop the session whenever they wanted with a hand signal to the examiner. Before the start of the recording, baseline anxiety was assessed by the State Trait Anxiety Inventory (Spielberg et al 1970) for state anxiety. A Visual Analogue Scale for anxiety (VAS-A), which describes the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable), was administered immediately before, after 10 minutes from the beginning and at the end of the session.
During the whole procedure, the examiner monitored on a computer screen the continuous recording of respiratory parameters breath by breath and interacted with the subjects only at standardized time intervals to administer the psychometric scales. Any disturbances like coughs, sneezes or laughs that could modify the respiratory pattern were noted by the examiner directly on the data file during the continuous recording, without interrupting the test.

### Assessment of respiratory physiology

Respiratory physiology was assessed by measuring the respiratory rate (RR) and the tidal volume (TV). For each respiratory parameter we calculated the mean, the average within-subject standard deviations (SDs), an indicator of the variability of the measure, and the Approximate Entropy index, an indicator of the irregularity and the "chaos" of the measure (Pincus 1991). The first 3 minutes of recording were discarded in order to minimize any possible influence

that familiarization with face mask and study apparatus could have on the respiratory pattern. Likewise, distortions during the breath by breath recording due to artifacts, like coughs, sneezes or laughs, were discarded.

## Statistical Analysis

### Approximate Entropy Index (ApEn)

To quantify the irregularity of each time series, we used the Approximate Entropy (ApEn) index, a model-independent statistic whose mathematical properties and biological applications have been described elsewhere (Pincus 1991; Pincus et al 1991). Briefly, the ApEn index is a nonnegative number assigned to a time series, with larger values corresponding to greater apparent process irregularity and smaller values corresponding to more instances of recognizable patterns in the data. Two input parameters, m and r, must be specified to compute ApEn: m measures the "length" of a sequence of contiguous observations (a run), and r measures the amount of noise in the data that is filtered out in the ensuing calculation. ApEn measures the likelihood that runs that are close (within r) for m observations remain close (within the same tolerance width r) when m is incremented. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and, conversely, random data (high irregularity) produces higher ApEn values. In this study, we calculated ApEn (m, r) values for all data sets using m=1 and r= 20% of the SD of the individual subject's time series. Normalizing r to each time series SD gives ApEn a translation- and scale-invariance. Computational aspects have been described in great detail by Pincus (Pincus 2000). Previous studies that included both theoretical analysis and clinical applications (Pincus et al 1993; Pincus et al 1996) have demonstrated that these input parameters produce good statistical validity (reproducibility) for ApEn applied to the time-series of the lengths considered here. We analyzed 17 minutes of recording with sampling rate=1, sample every 5 seconds and a typical sequence of data of approximately 200 data points.

ApEn index has been widely applied in endocrine studies (Charmandari et al 2002), heart rate studies (Pincus et al 1991) and respiratory physiology studies (Engoren 1998). Recently, it has been applied for analyzing data from psychiatric populations too (Caldirola et al 2004; Yeragani et al 2000; Yeragani et al 2002).

### Average within-subject standard deviation (SDs)

To quantify the overall variability of each measured parameter, we used the average within-subject standard deviation (SDs). SDs measures the magnitude of the deviation from the mean value for each parameter in each subject. In summary, SDs and ApEn values quantify two different characteristics of time-series data and provide complementary information. SDs describes the overall variability of a parameter over a period of time, whereas ApEn describes the dynamic pattern of that variability. For instance, tracings of a physiologic parameter with similar overall variability (SDs) might have a regular pattern over time (low ApEn), indicating low complexity of the system, or, on the contrary, an irregular pattern (high ApEn), indicating higher complexity of the system (Pincus 1994).

### Data analyses

Parametric statistical analyses were employed. The continuous data were analyzed by t-test, Analysis of Variance (ANOVA) and Multivariate Analysis of Covariance (MANCOVA). The nominal data were compared by chi-square analysis and Fisher exact test. The correlation between variables was tested using Pearson Correlation Coefficient.

## Results

The age of onset and the illness duration of our sample of patients were 28.3 ± 9.9 and 5.8 ± 7.0 years, respectively. Thirty-nine (67%) patients were agoraphobic. PASS (Panic Associated Symptoms Scale) total score was 6.9 ± 3.9 and PASS-PA (Panic Attacks), PASS-AA (Anticipatory Anxiety), PASS-AGO (Agoraphobia) subscales scores were 3.5 ± 2.4, 2.7 ± 1.9, 0.7 ± 0.9, respectively. FQ (Fear Questionnaire) total score was 40.9 ± 24.9 and FQ-AGO (Agoraphobia), FQ-BI (blood-injury phobia) and FQ-SOC (social phobia) subscales scores were 13.6 ± 12.9, 18.1 ± 11.8, 11.2 ± 8.0, respectively. Patients with PD had significantly higher STAI-I (State Trait Anxiety Inventory for state anxiety) scores before respiratory physiology assessment (45.7 ± 11.3) than healthy controls (29.2 ± 4.1), ($F= 61.7$, $fd = 1, 87$, $p<0.01$). VAS-A (Visual Analogue Scale for Anxiety) scores pre-Respiratory Assessment (RA), during-RA and post-RA in patients with PD and in healthy controls were respectively 35.9±25.6, 30.8±26.6, 24.1±23.4 and 7.0±8.7, 4.1±5.6, 2.7±4.7. ANOVA for repeated measures showed significant Diagnosis (D) ($F= 39.2$, $df= 1, 87$, $p<0.01$) and Time (T) ($F=$

9.2, df= 2, 174, $p<0.01$) effects for VAS-A scores, while no significant effect of TxD interaction was found.

There were no significant differences for gender distribution, age, weight, height, and Body Mass Index (BMI) between the two groups (Table1).

Among patients, the smokers and the non-smokers were respectively 25 (43.1%; 12 females and 13 males) and 33 (56.9%; 16 females and 17 males), whereas among healthy subjects they were respectively 9 (29%; 6 females and 3 males) and 22 (71%; 10 females and 12 males); this difference was not statistically significant. There were no significant differences for age of onset of smoking and smoking duration between the two groups, whereas the daily cigarettes consumption was significantly higher among patients than healthy subjects (Table1). Ten patients (40%) and 1 (11.1%) healthy subject fullfilled the DSM-IV criteria for nicotine dependence; this difference was not statistically significant.

The onset of smoking preceded the onset of PD in 22 patients (88%), whereas in 3 patients (12%) it came after the onset of PD.

## Respiratory physiology

MANCOVA with STAI scores as covariates showed no significant differences for the mean values and the average within-subject standard deviations (SDs) of the Respiratory Rate (RR) and Tidal Volume (TV) between patients with PD and healthy controls (RR=16.5±3.9 and 16.3±3.7, respectively; TV=0.52±0.2 and 0.51±0.2, respectively; SDsRR=2.5±1.3 and 2.1±0.8, respectively; SDsTV=0.16±1.1 and 0.21±0.1, respectively). MANCOVA with STAI scores as covariates and Gender (G) and Diagnosis (D) as grouping factors showed significantly higher Approximate Entropy (ApEn) indices of baseline respiratory parameters in patients with PD than in healthy controls (R=6.6, fd= 2, 83, $p<0.01$) but did not show either significant Gender effect or DxG interaction for the ApEn indices. Post-hoc Duncan comparisons showed significantly higher ApEn indices in patients with PD than in healthy controls for both RR and TV ($p<0.01$) (ApEn RR=1.37±0.3 and 1.13±0.2, respectively; ApEn TV=1.28±0.2 and 1.11±0.2, respectively).

Similar results were obtained including VAS-A scores as covariates in MANCOVA (data not shown).

Smoking

(1) Among patients, smokers and non-smokers did not differ for the mean values and the average within-subject standard deviations (SDs) of the respiratory parameters (Table 2), whereas smokers showed higher ApEn indices of baseline respiratory parameters than non smokers ($R=5.4$, $fd= 2, 55$, $p<0.01$). Post-hoc Duncan comparisons showed significantly higher ApEn indices in smokers than non-smokers for both RR and TV (Table 2). MANOVA with Gender (G) and Smoking (S) as grouping factors showed a Smoking effect ($R=5.2$, $df=2,53$, $p<0.01$) but did not show either significant Gender effect or SxG interaction for the ApEn indices (data not shown).
The smokers and non-smoker patients did not differ for anxiety levels before, during and after respiratory physiology assessment, measured by STAI-I and VAS-A scores (STAI-I scores were respectively 44.9±9.6 and 46.3±12.5; VAS-A scores pre-Respiratory Assessment (RA), during-RA and post-RA were respectively 34.4±23.9, 29.8±25.4, 24.2±24.6 and 36.9±27.1, 31.6±27.9, 24.1±22.8).
Linear Pearson Correlation did not show any significant correlation between ApEn indices of both RR and TV and the number of cigarettes per day or the smoking duration. Dependent and non- dependent patients did not differ for ApEn indices of both RR and TV.

(2) Among healthy subjects, smokers and non-smokers did not differ either for the mean values and the SDs (data not shown) or the ApEn indices of the respiratory parameters (ApEn RR=1.15±0.1 and 1.12±0.2, respectively; ApEn TV=1.08±0.2 and 1.12±0.2, respectively).

(3) MANOVA showed significant differences for the ApEn indices ($R=7.79$, $fd=4, 170$, $p<0.01$) and for the SDs ($R=2.67$, $fd=4, 170$, $p<0.05$) of the respiratory parameters among the smoker patients, non-smoker patients and healthy controls groups, whereas the mean values did not differ among the three groups. Post-hoc Duncan comparisons showed that the smoker patients had higher ApEn indices of RR and TV than both non-smoker patients and healthy controls, and the non-smoker patients had higher ApEn indices of RR and TV than healthy controls (Table 3). Post-hoc Duncan comparisons showed that smoker patients had higher SDs of RR than both non-smoker patients ($p<0.05$) and healthy controls ($p<0.05$) (SDsRR=2.9±1.6, 2.2±0.9 and 2.1±0.8, respectively), and higher SDs of TV than healthy controls ($p<0.05$) (SDsTV=0.2±0.1 and 0.1±0.5, respectively).

## Respiratory disorders

The lifetime prevalence of respiratory disorders was higher in patients than in healthy subjects (Fisher test $p<0.05$): 15 patients (25.9%) had lifetime respiratory disorders and 43 (74.1%) did not, whereas 2 healthy controls (6.4%) had lifetime respiratory disorders and 29 (93.6%) did not. Six patients (40%) had suffered from asthma, 2 (13.3%) from asthmatic bronchitis, 5 (33.4%) from bronchitis and 2 (13.3%) from pneumonia; none of the patients had ever suffered from tuberculosis or emphysema. One healthy subject had suffered from asthma and 1 from bronchitis. The distribution of the lifetime respiratory disorders did not differ between the smoker and non smoker patients; in the former group, 5 patients (20%) had lifetime respiratory disorders whereas 20 (80%) did not, and in the latter 10 patients (30.3%) had lifetime respiratory disorders whereas 23 (69.7%) did not. The onset of the respiratory disorder (mean age 14.6±11.8) preceded the onset of PD in 14 patients (93.3%), whereas in 1 patient (6.7%) it came after the onset of PD.

MANOVA with Smoking (S) and Respiratory Disorders (RD) as grouping factors showed a significant Smoking effect for the ApEn indices of the respiratory parameters ($R=5.4$, $df=2, 53$, $p<0.01$), whereas no significant effects of RD or SxRD interaction were found. MANOVA with Smoking (S) and Respiratory Disorders (RD) as grouping factors did not show either significant S, RD effects or SxRD interaction for the mean values and the SDs of the respiratory parameters (data not shown).

## Clinical Symptomatology

Smoker patients showed higher PASS total score (8.1 ± 4.3) than non smoker patients (5.4 ± 2.9) ($t= 2.8$, $p<0.01$) and higher PASS-PA score (4.3 ± 2.7) than non smoker patients (2.7 ± 1.7) ($t= 2.7$, $fd=56$, $p<0.01$), whereas the two groups did not significantly differ for PASS-AA and PASS-AGO subscales scores (PASS-AA=3.2 ± 1.9 and 2.4 ± 1.8 respectively; PASS-AGO=0.6 ± 1.0 and 0.7 ± 0.9 respectively) and for FQ total and subscale scores (FQ TOT=37.4 ± 23.2 and 43.9 ± 26.3 respectively; FQ AGO=12.3 ± 9.6 and 14.6 ± 14.9 respectively; FQ BI=16.0 ± 12.5 and 19.7 ± 11.2 respectively; FQ SOC=10.5 ± 8.5 and 11.6 ± 7.7 respectively). Dependent smoker patients showed higher PASS-PA score (6.0 ± 3.4) than non-dependent smoker patients (2.8 ± 1.8) ($t= 2.7$, $fd=56$, $p<0.05$), whereas the two groups did not significantly differ for the other PASS scores and for FQ total and subscale scores (data not shown).

## Discussion

Our study showed that (1) the patients with PD have a higher irregularity (higher ApEn indices) in their baseline respiratory patterns than the healthy subjects; (2) smoking is associated with a higher irregularity in the baseline respiratory patterns in patients with PD, whereas in healthy subjects is not; (3) the respiratory disorders do not seem to account for the influence of smoking on the irregularity of respiratory patterns; (4) the smoker patients have a higher severity of the panic attacks than the non-smokers.

### (1) Respiratory irregularity in PD

Our study found significantly greater levels of irregularity and "chaos" of the baseline respiratory patterns in patients with PD than in healthy subjects, extending our previous report (Caldirola et al 2004; Yeragani et al 2002). Baseline anxiety, anxiety during the procedure and individual variables were unable to explain the results. The greater respiratory irregularity in patients with PD might indicate an instability state in their respiratory function, on which external/internal perturbations might impact leading to panic attacks (Caldirola et al 2004).

### (2) Smoking and respiratory irregularity

In our sample the proportion of smokers in patients with PD is not significantly higher than in healthy subjects, whereas in previous studies it was (Amering et al 1999; Breslau et al 1991; Pohl et al 1992). This difference could depend on the inclusion in our study of the active regular smokers only; moreover, our data are not completely comparable with epidemiologic reports from general populations since they are obtained from a clinical sample. The age of onset of smoking preceded the onset of PD in most of the patients, in agreement with the reported unidirectional association between smoking and panic (Amering et al 1999; Breslau and Klein 1999; Isensee et al 2003; Pohl et al 1992). Smoker patients show higher respiratory irregularity than non-smoker patients, whereas smoker and non-smoker healthy subjects do not differ in their respiratory patterns; however, since the subgroup of non-smoker healthy subjects is small we cannot exclude a possible type II error in the comparison of smoker and non-smoker healthy subjects. Finally, both smoker and non-smoker patients show higher respiratory irregularity than healthy subjects. These differences are not explained by the anxiety levels, the number of daily smoked cigarettes or by gender. A possible influence of nicotine withdrawal cannot

be excluded, even if the lack of differences between smoker and non-smoker patients for the baseline and procedural anxiety does not support this possibility. Contrary to ApEn indices, the mean and SDs values of the respiratory parameters did not discriminate between groups. Our findings support the idea that the link between smoking and panic may involve a smoking influence on respiratory function. The higher respiratory irregularity in smokers patients suggests an impairing effect of smoking on the respiratory function in PD. The lack of smoking influence on the respiratory patterns regularity in the healthy subjects may suggest a specific smoking influence on a vulnerable respiratory function in PD. Finally, the finding that also non-smoker patients have a higher respiratory irregularity than healthy subjects suggests that smoking does not account for all the differences between patients and healthy controls, supporting the idea that the respiratory irregularity might be an intrinsic feature of patients with PD. Smoking might act as "disrupting" factor specifically on the baseline respiratory instability of patients with PD, possibly influencing the onset and/or the maintenance of the disorder. Whether higher respiratory irregularity could be a state feature of patients with PD or a trait marker of vulnerability to PD is not clarified yet. The higher irregularity in baseline respiratory patterns in children of patients with PD (Perna et al 2002) and the abnormal respiratory patterns in healthy first-degree relatives of patients with PD (Coryell et al 2001) might support the latter hypothesis.
The influence of smoking on the respiratory irregularity in PD could arise from multiple mechanisms. (a) From nicotine effects on the release/metabolism of several neurotransmitters that modulate the respiratory function (Berlin et al 1995; Haji et al 2000; Seth et al 2002; Vizi and Lendvai 1999; Wonnacot et al 1989); (b) from nicotine effects on selective nicotinic cholinergic receptors (nAChRs) in central and peripherical sites involved in respiratory modulation (Choen et al 2002); (c) from a direct nicotine modulation of respiratory pattern by affecting the excitatory neurotrasmission in brainstem respiratory pace-makers neurons (Shao and Feldman 2001; Shao and Feldman 2002); finally, (d) from possible effects of other substances in cigarette smoke, such as carbon monoxide (Breslau and Klein 1999).

### (3) Smoking and respiratory disorders

Patients with PD show a significantly higher lifetime prevalence of respiratory disorders (RD) than healthy subjects; the age of onset of the RD preceded the onset of PD in most of the patients. These results are in agreement with previous studies, even if the proportion of RD in our

sample is lower (Goodwin and Pine 2002; Goodwin et al 2003; Perna et al 1994; van Beek et al 2003; Verburg et al 1995). This difference could depend on the exclusion from our study of subjects with significant concurrent RD and the use of direct medical interview instead of self-reports; moreover, our data are not completely comparable with epidemiologic reports from general populations since they are obtained from a clinical sample. Our results show that RD per se did not influence the respiratory irregularity and that smoker patients with and without RD did not differ in the irregularity of their respiratory patterns. However, since the compared subgroups are very small we cannot exclude a type II error. Keeping in mind this limitation, RD does not seem to account for the influence of smoking on the irregularity of respiratory function in PD, even if a possible role of subclinical respiratory impairments cannot be excluded. Our results seem to support a possible direct effect of smoking on PD (Breslau and Klein 1999) through an influence on the respiratory irregularity regardless of the RD. However, the idea of an indirect effect of smoking by the RD (Breslau and Klein 1999) cannot be completely excluded because other different mechanisms than the respiratory irregularity could be involved.

(4) Clinical symptomatology

Smoker patients show a higher severity of panic attacks than non-smokers, whereas the two groups do not differ for the severity of the other clinical features of PD. The comparison of dependent and non-dependent patients show similar results. Since panic attacks are considered the "core" symptoms of the PD linked to an abnormal regulation of the respiratory system (Bellodi and Perna 1998; Klein 1993), this finding parallels the found smoking effect on the respiratory function. However, a role of panic like symptoms induced by nicotine through other pathways than respiration, such as via norepinephrine/catecholamine release (Vizi and Lendvai 1999; Isensee et al 2003), cannot be excluded.

In conclusion, our study suggests that a possible link between smoking and panic may involve a direct influence of smoking on the respiratory function. Smoking increases the irregularity in the baseline respiratory patterns of the patients with PD and might act as "critical factor" influencing the onset and/or the maintenance of the disorder. The main limitations of the study are the possible type II errors arisen from the small size of the compared subgroups. Further studies with larger sample will be necessary to confirm our results.

## Future prospects

Till now, the overall available data support the axiogenic properties of smoking in PD whereas the possible ansiolitic effects seem less plausible. In agreement with this idea, a high number of patients reduce or quit smoking because of their PD and a higher number of successful attempts to stop smoking in PD than in other anxiety disorders was found (Amering et al 1999; Lopes et al 2002); however, an exhaustive evaluation of the effect of quitting smoking on the clinical symptomatology has not been made yet. Longitudinal studies will be necessary to investigate the possible positive effects of quitting smoking on respiratory function and clinical symptomatology

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Table 1. Demographic and epidemiological characteristics of the sample.

| | Patients with PD (n=58) | Healthy Subjects (n=31) | P |
|---|---|---|---|
| Age (years) | 33.8 (11.4) | 33.0 (8.2) | ns |
| Sex (females) | 29 (50%) | 16 (52%) | ns |
| Weight (Kg) | 64.6 (11.8) | 68.9 (11.6) | ns |
| Height (m) | 1.69 (0.1) | 1.72 (0.1) | ns |
| Body Mass Index (Kg/m2) | 22.3 (2.8) | 23.1(2.9) | ns |
| Smokers | 25 (43.1%) | 9 (29%) | ns |
| N° cigarettes per day | 14.3 (9.2) | 6.6 (4.8) | <0.05 |
| Onset of smoking (years) | 17.4 (4.2) | 18.2 (4.3) | ns |
| Smoking duration (years) | 16.1 (12.6) | 20.2 (11.8) | ns |

Values are expressed as mean (SD) and number (%)

Table 2. Mean values, within-subject standard deviations (SDs) and Approximate Entropy (ApEn) indices* of respiratory parameters in PD group.

| | Smoker patients (n=25) | Non-smoker patients (n=33) | p |
|---|---|---|---|
| RR (breath min-1) | 17.5 (4.5) | 15.8 (3.3) | ns |
| TV (l min-1) | 0.52 (0.2) | 0.52 (0.2) | ns |
| SDs RR | 2.9 (1.6) | 2.2 (0.8) | ns |
| SDs TV | 0.2 (1.1) | 0.1 (0.1) | ns |
| ARR | 1.50 (0.2) | 1.28 (0.3) | $p<0.01$ |
| ATV | 1.36 (0.2) | 1.22 (0.2) | $p<0.05$ |

Values are expressed as mean (SD)
RR, respiratory rate; TV, tidal volume; SDs, average within-subject standard deviations.
ARR, ApEn of respiratory rate; ATV, ApEn of tidal volume.
* Larger values correspond to greater irregularity in the process

Table 3. Approximate Entropy (ApEn) indices* of respiratory parameters in PD and healthy subject groups.

| | Smoker Patients (n=25) | Non-smoker patients (n=33) | Healthy subjects (n=31) |
|---|---|---|---|
| ARR | 1.50 (0.2) a | 1.28 (0.3) b | 1.13 (0.2) c |
| ATV | 1.36 (0.2) d | 1.22 (0.2) e | 1.11 (0.2) f |

Values are expressed as mean (SD)
ARR, ApEn of respiratory rate; ATV, ApEn of tidal volume.
$a > b$, $p<0.01$; $a > c$, $p<0.01$; $b > c$, $p<0.05$;
$d > e$, $p<0.05$; $d > f$, $p<0.01$; $e > f$, $p<0.05$;
* Larger values correspond to greater irregularity in the process

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# Chapter 8

## Paroxetine and respiration in Panic Disorder: a preliminary study

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## Abstract

The biological mechanisms underlying the efficacy of SSRIs in Panic Disorder (PD) are unknown. Since PD is characterized by an abnormal respiratory function, the antipanic effect of SSRIs could be linked to their influence on the respiratory function. We evaluated the influence of one week paroxetine treatment on baseline respiratory patterns in 15 patients with PD. The baseline respiratory patterns assessment was carried out using a breath by breath Quarkb2 stationary testing system. Respiratory pattern irregularity was measured by applying the Approximate Entropy Index (ApEn). A significant decrease of the ApEn indices of the respiratory parameters after one week of treatment was found, indicating a decrease of irregularity and "disorder" in respiratory patterns. An influence on the respiratory function by serotonergic system might be an important mechanism of the anti-panic effect of paroxetine and the decrease of respiratory irregularity might express a "normalization" of the abnormal respiratory function underlying PD.

## Introduction

Serotonin Selective Re-uptake Inhibitors (SSRIs) are effective in decreasing clinical symptomatology and carbon dioxide ($CO_2$) hyperreactivity in patients with Panic Disorder (Ballenger et al 1998; Bertani et al 1997; Lecrubier et al 1997; Lecrubier and Judge 1997; Perna et al 2002; Perna et al 2001). SSRIs are able to significantly reduce the $CO_2$ hyperreactivity after the first week of treatment (Bertani et al 1997; Perna et al 2002; Perna et al 1997) and this might be a significant predictor for a good clinical outcome after one month of treatment (Bertani et al 1997; Perna et al 2002; Perna et al 1997). The biological mechanisms underlying the antipanic effect of SSRIs are still unclear. Since PD is characterized by an abnormal respiratory function (Bellodi and Perna 1998; Gorman et al 1988; Klein 1993; Klein 1994; Martinez et al 1996; Papp et al 1993; Papp et al 1997) and the serotonergic system modulates the respiratory control mechanisms (Bianchi et al 1995; Haji et al 2000; Lundberg et al 1980;

McCrimmon et al 1995), the antipanic effect of SSRIs could be linked to their influence on the respiratory function. The finding that anti-panic drugs reduce the ventilatory response to $CO_2$ in patients with PD (Bocola et al 1998; Gorman et al 1997; Haji et al 2000; Pols et al 1993) might supports their direct influence on respiratory control mechanisms, even if alternative explanations cannot be excluded. Limbic system and fear generating circuits might be involved in the pathophysiology of PD (Gorman et al 2001; Sinha et al 2000) and thus the antipanic effect of SSRIs might arise from modulating these circuits with an indirect effect on respiration (Balaban and Thayer 2001; Hashimoto et al 1996; Loewy 1990; Stutzmann and LeDoux 1999). Data from available studies are not informative enough to clarify this issue also because the findings are conflicting. Animal studies showed a marked decrease of $CO_2$-induced tachypnea after fluoxetine and paroxetine treatments and an increased respiratory variability after serotonin depletion, suggesting a direct respiratory effect of SSRI regardless of their anxiolitic effect (Annerbrink et al 2003; Henderson et al 1999; Olsson et al in press). On the other hand, SSRIs treatment did not significantly modify baseline respiratory variability of a sample of patients with PD (Martinez et al 2001). The goal of this study is to investigate the influence of paroxetine, the most powerful inhibitor of serotonin (Hyttel 1994; Johnson 1992; Thomas et al 1987), on baseline respiratory function in PD. Since patients with PD are characterized by higher irregularity of their baseline breathing patterns (Abelson et al 2001; Caldirola et al in press-a; Wilhelm et al 2001a; Wilhelm et al 2001b; Yeragani et al 2002a), we evaluated the influence of one week paroxetine treatment on baseline breathing patterns in PD. We applied the Approximate Entropy index (ApEn), a non linear measure of irregularity, since non-linear methods are considered the most proper way to analyse the complexity of the physiological functions (Pincus et al 1991a).

## Methods

### Subjects

Fifteen outpatients with PD with/without Agoraphobia were included in the study. They were recruited among those consecutively referred to the Anxiety Disorders Clinical and Research Unit of San Raffaele Hospital, Milan, over a period of 3 months. Psychiatric diagnosis was obtained by the MINI International Neuropsychiatric Interview-Plus for DSM IV (Sheehan et

al 1994). Concurrent psychiatric disorders, except specific phobias, were exclusion criteria for patients with PD. At the beginning and after one week of paroxetine treatment the severity of clinical symptomatology in patients with PD was measured by the Panic Associated Symptoms Scale (PASS), which assesses panic attacks (PASS-PA subscale), anticipatory anxiety (PASS-AA subscale) and agoraphobia (PASS-AGO subscale) (Argyle et al 1991), and the Fear Questionnaire (FQ) which assesses agoraphobia, blood-injury phobia and social phobia (Marks and Mathews 1979). Exclusion criteria for all subjects were significant concurrent cardio-circulatory and respiratory diseases, significant hypertension (systolic > 180 mm Hg, diastolic > 100 mm Hg), pregnancy or epilepsy, according to a direct physical examination and medical history. Subjects with respiratory diseases requiring concurrent treatment were also excluded.

### Drug treatment

Since initial high doses or rapid increases of SSRIs could enhance the anxiety levels during the first days of treatment in PD (Den Boer and Westenberg 1988; Humble and Wistedt 1992), in this study we used low doses of paroxetine (10 mg/day) for the entire week. No concomitant psychotropic drugs or psychotherapeutic interventions were allowed, except for standardized information on PD given to all the patients at the beginning of the study. An open study design was applied. Before the first respiratory assessment, subjects had to have been off all psychotropic medications for at least 2 weeks. None of the patients took fluoxetine in the 6 months before. Eight patients had discontinued low doses of benzodiazepines, whereas 7 had not taken any drugs.

### Respiratory assessment

The respiratory assessment (RA) was performed before the beginning of the treatment (Day 0) and after one week (Day 7). The same standardized procedure was applied.

### Apparatus: the Quark b2 stationary testing system

The Quark b2 stationary testing system (Cosmed, Rome, Italy) allows assessment of respiration physiology by monitoring respiratory function and pulmonary gas exchange on a breath by breath basis during natural breathing of resting subjects. The breath by breath recording by the Quark b2 system is widely used in sports medicine and respiration physiology studies,

in accordance with the recommendations of the American Thoracic Society and the European Respiratory Society (Palange et al 2000; Schena and Padoin 1999).
The Quark b2 system consists of a mobile unit containing the principal components (infrared light turbine, gas analyzers, electronic sensors) connected on-line to a computer to allow continuous breath by breath recording of respiratory parameters. Before each test, the turbine and the analyzers were calibrated in order to maintain optimal technical characteristics of the apparatus. An open light face mask connects the subject to the respiratory testing system.

### Procedure

Since many substances could affect respiratory patterns (Akiyama and Kawakami 1999b), the subjects were asked to refrain from alcohol for at least 36 hours, from beverages or food containing xanthines for at least 8 hours, from non-steroid anti-inflammatory drugs for at least 36 hours and from any food or smoking for at least 2 hours before the respiratory physiology assessment.
Recording of respiratory parameters was carried out by medical doctors trained in the use of the Quark b2 system. A standardized procedure was used throughout to minimize any confounding influences (Akiyama and Kawakami 1999a) as described in details elsewhere (Caldirola et al submitted; Caldirola et al in press-a). The recording was carried out in a quiet room and took 20 minutes. Patients were recorded between 4 p.m. and 6 p.m. to avoid biases related to circadian rhythms of respiratory control (Spengler et al 2000; Stephenson et al 2000). Before the recording started, subjects rested for 20 minutes and were familiarized with the study apparatus.
Before the start of the recording, baseline anxiety was assessed by the State Trait Anxiety Inventory (STAI) (Spielberg et al 1970) for state anxiety. A Visual Analogue Scale for anxiety (VAS-A), which describes the degree of global subjective anxiety on a continuum from 0 (no anxiety) to 100 (the worst anxiety imaginable), was administered immediately before, after 10 minutes from the beginning and at the end of the respiratory assessment.

### Assessment of respiratory physiology

Respiratory physiology was assessed by measuring the respiratory rate (RR) and the tidal volume (TV). For each respiratory parameter we calculated the mean, the average within-subject

standard deviations (SDs), an indicator of the variability of the measure, and the approximate entropy index, an indicator of the irregularity and the "disorder" of the measure (Pincus 1991). The first 3 minutes of recording were discarded in order to minimize any possible influence that familiarization with face mask and study apparatus could have on the respiratory pattern. Likewise, distortions during the breath by breath recording due to artifacts, like coughs, sneezes or laughs, were discarded.

## Statistical Analysis

### Approximate Entropy Index (ApEn)

To quantify the irregularity of each time series, we used the Approximate Entropy (ApEn) index, a model-independent statistic whose mathematical properties and biological applications have been described elsewhere (Pincus 1991; Pincus et al 1991b). Briefly, the ApEn index is a nonnegative number assigned to a time series, with larger values corresponding to greater apparent process irregularity and smaller values corresponding to more instances of recognizable patterns in the data. Two input parameters, m and r, must be specified to compute ApEn: m measures the "length" of a sequence of contiguous observations (a run), and r measures the amount of noise in the data that is filtered out in the ensuing calculation. ApEn measures the likelihood that runs that are close (within r) for m observations remain close (within the same tolerance width r) when m is incremented. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and, conversely, random data (high irregularity) produces higher ApEn values. In this study, we calculated ApEn (m, r) values for all data sets using m=1 and r= 20% of the SD of the individual subject's time series. Normalizing r to each time series SD gives ApEn a translation- and scale-invariance. Computational aspects have been described in great detail by Pincus (Pincus 2000). Previous studies that included both theoretical analysis and clinical applications (Pincus et al 1993; Pincus et al 1996) have demonstrated that these input parameters produce good statistical validity (reproducibility) for ApEn applied to the time-series of the lengths considered here. We analyzed 17 minutes of recording with sampling rate=1, sample every 5 seconds and a typical sequence of data of approximately 200 data points.

ApEn index has been widely applied in endocrine studies (Charmandari et al 2002; Roelfsema

et al 2002; van den Berg et al 1997), heart rate studies (Pincus et al 1993; Pincus et al 1991b; Pincus and Viscarello 1992) and respiratory physiology studies (Engoren 1998). Recently, it has been applied for analyzing data from psychiatric populations too (Caldirola et al in press-b; Kaloupek et al 2000; Yeragani et al 2000; Yeragani et al 2002b).

### Average within-subject standard deviation (SDs)

To quantify the overall variability of each measured parameter, we used the average within-subject standard deviation (SDs). SDs measures the magnitude of the deviation from the mean value for each parameter in each subject.

In summary, SDs and ApEn values quantify two different characteristics of time-series data and provide complementary information. SDs describes the overall variability of a parameter over a period of time, whereas ApEn describes the dynamic pattern of that variability. For instance, tracings of a physiologic parameter with similar overall variability (SDs) might have a regular pattern over time (low ApEn), indicating low complexity of the system, or, on the contrary, an irregular pattern (high ApEn), indicating higher complexity of the system (Pincus 1994).

### Data analyses

Non-parametric statistical analyses were employed since the data were not normally distributed. The continuous data were analyzed by the Wilcoxon matched pairs test and the Mann-Whitney U test. The correlation between variables was tested using the Spearman Correlation Test.

## Results

Ten females (67%) and 5 males (33%) were included in the study. Eleven patients (73%) were agoraphobics. The mean age, the age of onset of PD and the illness duration were 30.0± 10.2, 23.5 ± 8.0 and 5.0 ± 4.8 years, respectively. The mean weight (Kg), height (m), and BMI (Kg/m2) were 65.9± 10.5, 1.71 ± 0.1 and 22.4 ± 2.9, respectively.

The mean and SDs values and the ApEn indices of the measured respiratory parameters at the beginning of the treatment are listed in Tables 1. Females and males did not significantly differ for mean, SDs and ApEn indices of the respiratory parameters (data not shown). No correlation between ApEn indices and weight, height, BMI, age and illness duration was found.

Wilcoxon matched pairs test showed no differences for the mean and SDs values of the respi-

ratory parameters across 1 week of paroxetine treatment, whereas a significant decrease of the ApEn indices of the parameters was found (Table 1). Females and males did not significantly differ for the ApEn indices at the end of the treatment (data not shown).
No significant differences were found comparing the baseline state anxiety, measured by STAI scores, and the anxiety during and at the end of the respiratory assessment, measured by VAS-A during RA and post-RA scores, on day 0 and 7; a significant decrease of the anxiety before starting the respiratory assessment, measured by VAS-A pre-RA scores, was found (Table 3).
No significant correlation between STAI scores, VAS-A pre, during and post RA scores and the ApEn indices of the respiratory parameters either on day 0 or day 7 was found. No significant correlation between the decrease of the ApEn indices, measured as Delta ApEn (values of ApEn indices on day 7 minus the values on day 0), and the decrease of the VAS-A pre RA scores, measured as Delta VAS-A pre (values of VAS-A pre RA on day 7 minus the values on day 0) was found; Delta ApEn of RR and TV and Delta VAS-A pre RA were -0.2 ± 0.2, -0.2 ± 0.4 and -19.1± 25.1, respectively.
The psychometrics scales scores at the beginning and the end of the treatment are listed in Table 3. A significant decrease of the PASS total (PASS-TOT) score and the PASS-AA subscale score across 1 week of paroxetine treatment was found, whereas no significant decrease of the other PASS subscales scores and total and subscales FQ scores was found (Table 3). No significant correlation between ApEn indices and psychometrics scales scores at the beginning and the end of the treatment was found; no significant correlation between the decrease of the ApEn indices (Delta ApEn) and the decrease of the TOT and AA subscale scores, measured as Delta TOT and Delta AA, was found. Delta TOT (scores of PASS-TOT on day 7 minus the scores on day 0) and Delta AA (scores of PASS-AA on day 7 minus the scores on day 0) were -3.1 ± 4.8, -1.3 ± 2.0, respectively.

## Discussion

The main finding of our study is that one week of treatment with paroxetine significantly decreases the ApEn indices of the respiratory parameters in patients with PD, indicating a decrease of irregularity and "disorder" in their respiratory patterns. On the contrary, the mean and SDs values did not significantly differ across the treatment. Our results are not explained by individual variables and gender difference. The main limitation of our study is the lack of a

comparison placebo-controlled group; therefore it is not completely certain whether the decrease of respiratory irregularity could be taken as indicator of a specific drug effect. However, most of baseline / procedural anxiety scores do not differ across the week of treatment and do not correlate with the ApEn indices; although the anxiety levels immediately before the respiratory assessment significantly decrease on day 7, a correlation between the reduction of the anxiety scores and the ApEn indices was not found. Therefore, our findings are not explained by anxiety levels or habituation to the procedure, even if other aspecific factors cannot be excluded. In agreement with previous studies, one week treatment with paroxetine did not improve the clinical symptomatology, except for a significant decrease of the anticipatory anxiety related to natural panic attacks (Bertani et al 2001; Perna et al 2001); this might be explained by an early anxiolitic effect of paroxetine or by the psychoeducational intervention at the beginning of the study. The dissociation between the respiratory irregularity and the clinical improvement, as well as the lack of correlation between the reduction of the anticipatory anxiety and the ApEn indices, do not support the idea that the decrease of respiratory irregularity could be secondary to clinical improvement. The decrease of the respiratory irregularity might arise from a paroxetine direct effect on the respiratory function. Our results are in agreement with previous findings showing that serotonergic manipulation affects respiratory variability in animals (Annerbrink et al 2003) and influences baseline ventilation in patients with PD but not in healthy subjects, regardless of their anxiety state (Kent et al 1996). Our results do not parallel those of Martinez and coworkers (31) but this probably arises from the differences in the study design and the used methods. Serotonergic system influences respiration by multiple pathways. Serotonergic neurons in the medullary raphe nuclei and the ventrolateral medulla have chemosensitive properties (Richerson et al 2001); raphe neurons project to phrenic and hypoglossal motoneurons as well as premotor respiratory neurons and respiratory rhythm generator in the braistem, suggesting a role of the serotonergic system in the modulation of both the respiratory rhythm and plasticity (Feldman et al 2003; Onimaru et al 1998; Pena and Ramirez 2002; Schwarzacher et al 2002); the serotonin transporter is expressed on human pulmonary membranes (Ramamoorthy et al 1993; Suharaa et al 1998) and the serotonergic peripherical activity modulates the bronchomotor tone and pulmonary blood flow (Dupont et al 1999; Eddahibi et al 2001; Lommel 2001; Szarek et al 1995). Finally, the modulation of serotonergic system improves the respiratory function in several respiratory

human disorders (Kraiczi et al 1999; Smoller et al 1998; Wilken et al 1997). In conclusion, the decrease of the respiratory irregularity supports the idea that an influence on the respiratory function by serotonergic system might be an important mechanism of the anti-panic effect of this drug and might express a "normalization" of the abnormal respiratory function underlying PD. However, since paroxetine modulates also the noradrenergic and cholinergic neurotransmission that are involved in respiratory regulation, its influence on the respiratory function could arise also from other pathways than the only serotonergic one (Haji et al 2000; Hyttel 1994; Thomas et al 1987). Because of the limitations discussed above and the small size of the sample, our results should be considered preliminary. Further studies should be performed in order to evaluate the changes of respiratory patterns across short / long term treatments with antipanic drugs and the relationship between the modulation of the respiratory patterns and the clinical outcome.

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Table 1. Mean, SDs and ApEn indices values of the respiratory parameters.

| | Day 0 | Day 7 | Wilcoxon test, p |
|---|---|---|---|
| RR (breath min-1) | 16.4 (5.2) | 16.1 (4.4) | 0.6 |
| TV (liters) | 0.6 (0.2) | 0.5 (0.2) | 0.5 |
| SDs RR | 2.6 (1.7) | 2.5 (1.7) | 0.9 |
| SDs TV | 0.2 (0.1) | 0.2 (0.1) | 0.7 |
| ARR | 1.44 (0.3) | 1.26 (0.3) | T=16, Z=2.5, p<0.05 |
| ATV | 1.27 (0.3) | 1.10 (0.3) | T=22, Z=2.1, p<0.05 |

Values are expressed as mean (SD)
RR, respiratory rate; TV, tidal volume; SDs, average within-subject standard deviations; ApEn, Approximate Entropy indices; ARR, ApEn of respiratory rate; ATV, ApEn of tidal volume.

Table 2. Anxiety levels before, during and at the end of the procedure.

| | Day 0 | Day 7 | Wilcoxon test, p |
|---|---|---|---|
| STAI | 47.7 (11.4) | 41.2 (14.1) | 0.1 |
| VAS-A pre RA | 41.5 (28.3) | 22.3 (21.0) | T=13.5, Z=2.6, p<0.01 |
| VAS-A during RA | 33.4 (30.2) | 28.8 (22.5) | 0.6 |
| VAS-A post RA | 29.5 (29.4) | 28.9 (24.5) | 0.8 |

Values are expressed as mean (SD)
STAI, State Trait Anxiety Inventory; VAS-A, Visual Analogue Scale for Anxiety; RA, Respiratory Assessment.

Table 3. Psychometric scale scores.

| | Day 0 | Day 7 | Wilcoxon test, p |
|---|---|---|---|
| PASS-TOT | 11.3 (6.2) | 8.2 (5.4) | T=11, Z=2.2, p<0.05 |
| PASS-PA | 4.8 (2.3) | 4.0 (2.7) | 0.2 |
| PASS-AA | 4.4 (2.4) | 3.1 (1.5) | T=8, Z=2.2, p<0.05 |
| PASS-AGO | 2.1 (3.1) | 1.1 (2.1) | 0.1 |
| FQ-TOT | 38.8 (24.7) | 37.8 (26.7) | 0.7 |
| FQ-AGO | 13.5 (11.6) | 14.3 (12.0) | 0.6 |
| FQ-BI | 14.2 (9.6) | 12.7 (11.1) | 0.3 |
| FQ-SP | 11.1 (9.9) | 10.9 (9.8) | 0.5 |

Values are expressed as mean (SD)

PASS-TOT, Panic Associated Symptoms Scale total score; PASS-PA, subscale assessing panic attacks; PASS-AA, subscale assessing anticipatory anxiety; PASS-AGO, subscale assessing agoraphobia; FQ-TOT, Fear Questionnaire total score; FQ-AGO, subscale assessing agoraphobia; FQ-BI, subscale assessing blood-injury phobia; FQ-SP, subscale assessing social phobia.

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# Summary and concluding remarks

Previous experimental studies have provided good evidence that a panic-respiration connection exists, but the nature of the respiratory abnormalities and the neuroanatomical machanisms are still unclear. This thesis has focused on the experimental study of the respiratory function in the pathophysiology and therapy of Panic Disorder (PD).

The study in chapter 2 has investigated the behavioural hyperreactivity to 35% $CO_2$ in patients with PD and Social Phobia (SP). Since these two disorders share many clinical, demographic and biological characteristics, we investigated the possibility that they share common underlying mechanisms. The results have shown that patients with SP have a stronger behavioural reactivity to $CO_2$ than that of healthy controls and similar to patients with PD. Thus, PD and SP might be two clinical syndromes that share common underlying pathogenetic mechanisms, indicated by similar vulnerability to $CO_2$ inhalation. However, the issue of SP is not completely clear-cut. Other studies have shown that subjects with SP have a higher behavioural reactivity to $CO_2$ with respect to that of healthy subjects but lower than patients with PD. Previous unpublished data from the group of Prof. Griez in Maastricht have shown that patients with SP are insensitive to 35% $CO_2$, whereas recent data from the same group have shown that patients with SP have a behavioural reactivity to 35% $CO_2$ between that of patients with PD and healthy subjects (Prof. Griez, personal communication). An explanation for these differences might be the possible heterogeneity of SP. Clinical syndromes that "on the surface" meet the DSM-IV diagnostic criteria for SP might arise from different underlying pathogenetic mechanisms. At least a subgroup of patients with SP, which share a behaviour hyperreactivity to $CO_2$ with patients with PD, might belong to the same spectrum of underlying "respiratory" vulnerability. According to this idea, they also might share the baseline respiratory irregularity with patients with PD. Further studies on the respiratory patterns of patients with SP could clarify whether the respiratory irregularity is an underlying feature of a "Panic-Phobic Spectrum".

Since $CO_2$ hyperreactivity is considered a biological marker of PD, a reduction of the $CO_2$ hyperreactivity might be an indication of the normalization of the pathogenetic mechanisms underlying PD. The study in chapter 3 has shown that the modulation of the serotonergic system, by one-week treatment with citalopram, is more effective in reducing 35% $CO_2$ hyperreactivity than a reboxetine treatment. This finding is consistent with the results of our recent study that have shown a significantly weaker reduction of panic attacks with a twelve-week reboxetine treatment than with paroxetine. On the contrary, the reduction of avoidance and

anticipatory anxiety was similar with both treatments. Overall, these findings suggest that the serotonergic system might specifically modulate the "core" phenomenon of PD, i.e. the panic attack, whereas the noradrenergic one might be more involved in clinical phenomena usually following the panic attacks, i.e. the anticipatory anxiety and the avoidant behaviors. Since the serotonergic system has a relevant role in the modulation of respiratory function, the antipanic effect of serotonergic drugs on both the $CO_2$-induced and naturally occurring panic attacks might be linked to their influence on respiratory control mechanisms.

This idea has been developed in the last study of this thesis (chapter 8) in which we investigated the influence of paroxetine on baseline respiratory function in patients with PD. One-week paroxetine treatment significantly decreases the irregularity and "chaos" in their baseline respiratory patterns. Since this effect precedes the reduction of clinical symptoms and has no correlations with anxiety levels, the possibility that the decrease of respiratory irregularity could be secondary to clinical improvement seems unlikely. The decrease of the respiratory irregularity might arise from direct effect of paroxetine on respiratory function. This supports the idea that an influence on the respiratory function might be an important mechanism of the serotonergic anti-panic drugs' effect and might indicate a "normalization" of a malfunction of the respiratory system underlying PD.

Chapters 4 to 7 are specifically focused on the experimental study of the features of the respiratory function in patients with PD. The study in chapter 4 illustrates that patients with PD have higher entropy in respiratory baseline patterns than healthy controls, indicating higher levels of irregularity and complexity in their respiratory function. This feature is not influenced by anxiety state or severity of illness. Entropy describes the amount of disorder in processes and systems. The higher entropy in the respiratory function of patients with PD might indicate an intrinsic instability in their respiratory homeostasis on which different critical inputs could act as "disruptive" factors leading to panic attacks. In order to test the idea that higher respiratory entropy might represent a vulnerability factor to panic, in the study in chapter 5 we investigated whether the baseline respiratory instability may underlie the behavioural hyperreactivity to hypercapnia in patients with PD. The results showed that patients who panicked during 35% $CO_2$ inhalation have higher baseline respiratory irregularity than both patients who did not panic and healthy subjects, and the higher irregularity of tidal volume is a respiratory predictor of induced panic. These findings support the idea that the behavioural

hyperreactivity to $CO_2$ might be linked to a malfunction of the respiratory system. Baseline chaotic breathing might affect the ability of patients to carry out adequate respiratory responses when changes occur. Since $CO_2$ inhalation induces a ventilatory response to restore homeostasis, the baseline respiratory irregularity might constrain efficient compensatory mechanisms during $CO_2$ inhalation, possibly influencing the occurrence of the induced panic attacks. These findings are consistent with the results of the study in chapter 6, in which we investigated the different types of dyspnea induced by 35% $CO_2$ inhalation in patients with PD. The Breathing Effort, which arises from the conscious awareness of muscular effort during activation of respiratory skeletal muscles, was the most peculiar dyspnea sensation in $CO_2$-induced panic attacks. The association between a panic response to $CO_2$ and the Breathing Effort may be explained by the abnormalities found in the respiratory function of patients with PD. Higher irregularity and instability in baseline breathing patterns might decrease the mechanical efficiency of the respiratory responses, leading to sense of Breathing Effort when respiratory stimuli occur, such as during $CO_2$ inhalation. The Sense of Suffocation, which arises from the activation of the chemoreceptors, was present in $CO_2$-induced panic attacks but this link disappeared after patients were subgrouped according to the similarity of $CO_2$-induced responses to their unexpected panic attacks. This findings suggests that while chemosensitivity is involved in $CO_2$ reactivity, it might not be central to unexpected panic attacks.
Daily smoking is associated with an increased risk for later onset of panic attacks or disorder, possibly by affecting the respiratory system function. In the study in chapter 7 we investigated the mechanisms linking smoking and respiratory function in PD. Smoking patients showed higher baseline respiratory irregularity than non-smoking patients, whereas smoking and non-smoking healthy controls did not differ in their respiratory patterns. The higher respiratory irregularity in smoking patients and the lack of influence of smoking on the respiratory patterns of healthy controls may suggest a specific influence of smoking on a vulnerable respiratory function in PD. The respiratory disorders did not account for the influence of smoking on respiratory irregularity, suggesting a direct impairing effect of smoking on the respiratory function in PD. Finally, the finding that non-smoking patients also have a higher respiratory irregularity than healthy subjects supports the idea that the respiratory irregularity might be an intrinsic feature of patients with PD. Smoking might be a "disruptive" factor specific to the baseline respiratory instability of patients with PD, possibly influencing the onset and/or the maintenance of the disorder.

Overall, the findings from this thesis indicate a key role of the respiratory function in the pathophysiology of PD, and support the idea that panic attacks might be specifically linked to a malfunction of the respiratory system rather than being simply equivalent to fear/stress reactions. The main conclusions are that 1) the baseline respiratory entropy might be an indication of respiratory malfunction and represent a vulnerability factor to panic. Instability in the respiratory system might lead to panic when the system fails to cope with external/ internal stimuli and to restore the state of equilibrium; 2) the improvement of the respiratory malfunction / instability might be a specific target of anti-panic drugs treatments. This idea should be tested by investigating the relationship between the modulation of the respiratory patterns across short / long term antipanic treatments and clinical outcome. The improvement of the respiratory instability might also be the target of non-pharmacological treatments. An ongoing study by our group is investigating the effect of breathing training, aimed at decreasing the respiratory irregularity, on respiratory patterns and clinical symptoms in patients with PD.

However, several critical issues are open and require clarification:

1) The source of the respiratory entropy, that could arise from multiple mechanisms. a) The respiratory entropy might be the indication of primary respiratory abnormalities. It might represent an adaptive/compensatory response to dysfunctional respiratory signals, such as chemoceptive inputs, or arise from an intrisic deranged activity in the brainstem respiratory network that shapes the respiratory patterns; b) the respiratory entropy might be explained by perturbations of other basic systems, since basic physiological functions in the organism are strictly interconnected in complex neural networks. Recent evidence has shown an abnormal regulation of cardiovascular and balance systems in patients with PD, suggesting the idea of a broader dysfunction of the neuronal circuits that regulate physiological homeostatic functions in PD; c) the respiratory entropy might originate from brain centers higher than the brainstem, such as from the limbic system that is involved in respiratory changes during emotional states. Although our studies have shown a lack of effect of anxiety levels on the baseline respiratory entropy, a possible influence of the emotional states on the respiratory patterns cannot be completely excluded. Further studies of the respiratory/cardiac/vestibular integrated patterns under different experimental manipulations might be useful to clarify these issues.

2) The issue of whether higher respiratory entropy might be a state feature of patients with PD or a trait marker of vulnerability to PD. Further familial studies should be performed to clarify this crucial matter.
3) The possible specific neuroanatomical circuits involved in "respiratory panic".
Neural maps, representing the internal organismal milieu, are assembled moment by moment in the Central Nervous System, to allow an organism's adaptation to internal/external changes or impending future events, in order to maintain bodily well-being. These basic regulatory processes take place continuously beyond consciousness and only occasionally pervade the conscious awareness as "primal emotions". Such emotional states, such as extreme thirst and air hunger, which arise from basic vegetative systems, might have the evolutionary role of signaling that the survival of the organism is threatened. Consistent evidence supports the view that primal emotions are subserved by similar complex circuits in phylogenetically ancient brain areas. In recent brain imaging studies, the activation/deactivation pattern during 5% and 8% $CO_2$ induced air hunger in healthy subjects was investigated. The consciousness of respiratory sensations involved a broad network of activation in the "ancient" brain including the brainstem, midbrain, the hypothalamus, limbic and paralimbic areas, hippocampus, parahippocampal and fusiform gyrus, anterior insula, anterior cingulate and cerebellum, whereas deactivation in the dorsal and posterior cingulate and the prefrontal cortex were found. These brain circuits include areas, such as the cerebellum, that are not involved in fear models and are not completely overlapping with the "fear network" circuits. This evidence supports the idea that the panic attacks could be different from fear reactions and might be the expression of "primal" emotions arising from specific phylogenetically ancient brain circuits processing physiological perceptions/sensations linked to homeostatic functions. Klein's "false suffocation alarm" might belong to the spectrum of the primal emotions. An abnormal processing of information about the organism's internal milieu in the network subserving basic emotions, could lead to an abnormal "respiratory primal emotion" to pervade the consciousness of patients as panic attack. The respiratory irregularity and possible abnormal signals from cardiac and vestibular systems might be the triggering inputs for the activation of these circuits in PD. However, these idea remains speculative since the available data are not sufficient. Further neuroimaging studies in patients with PD might provide room for testing these ideas.

# Samenvatting en conclusie

Dit proefschrift richtte zich op het experimenteel onderzoek van de ademhaling in de pathofysiologie en therapie van de paniekstoornis (PD).
In het onderzoek in hoofdstuk 2 evalueerden wij de overgevoeligheid van patiënten met paniekstoornis en sociale fobie (SP) voor 35% $CO_2$, teneinde de mogelijkheid te onderzoeken dat beide stoornissen gemeenschappelijke onderliggende mechanismen zouden hebben. De resultaten toonden aan dat patiënten met SP een grotere gevoeligheid voor $CO_2$ tonen in vergelijking met gezonde controlepersonen, evenals PD patiënten een overgevoeligheid voor $CO_2$ tonen. Derhalve zouden PD en SP twee klinische syndromen kunnen zijn met gemeenschappelijke onderliggende pathogenetische mechanismen, blijkens hun gemeenschappelijke kwetsbaarheid voor de $CO_2$ inhalatie. Echter, het vraagstuk van de SP is complexer dan dat. Andere onderzoeken hebben aangetoond dat patiënten met SP sterker op $CO_2$ reageren dan gezonde controles, maar minder sterk dan PD patiënten. Een verklaring voor deze verschillende bevindingen zou kunnen liggen in de mogelijke heterogeniteit van de SP. Klinische syndromen die "oppervlakkig" gezien voldoen aan de DSM-IV diagnostische criteria voor SP zouden kunnen voortkomen uit verschillende onderliggende pathogenetische mechanismen. Tenminste een subgroep van patiënten met SF, die de overgevoeligheid voor de $CO_2$ challenge delen met de PD patiënten, zouden kunnen behoren tot hetzelfde spectrum van onderliggende "respiratoire" kwetsbaarheid. In deze visie zouden deze patiënten ook de baseline respiratoire onregelmatigheden delen met de PD patiënten. Verdere studies naar ademhalingspatronen bij SP patiënten zou helderheid kunnen brengen in de vraag of onregelmatige ademhaling een kenmerk is van een "paniek-fobisch spectrum".
Omdat $CO_2$ overgevoeligheid beschouwd wordt als een biologische marker voor PD, zou een afname van deze gevoeligheid een indicatie kunnen zijn van van de normalisatie van de onderliggende pathogene mechanismen in PD. De studie in hoofdstuk 3 beschreef dat de modulatie van het serotonerge systeem middels behandeling gedurende een week met citalopram effectiever is op afname van de $CO_2$ overgevoeligheid dan een week reboxetine behandeling. Gezien de rol van het serotonerge systeem in de modulatie van de ademhaling, zou het anti-paniek effect van serotonerge medicatie verbonden kunnen worden aan hun invloed op respiratoire controle-mechanismen.
Dit idee werd verder ontwikkeld in het laatste hoofdstuk van dit proefschrift (hoofdstuk 8) waar het effect van paroxetine op baseline ademhaling van PD patiënten beschreven werd.

Een week behandeling met paroxetine resulteerde in een significante afname van de onregelmatigheid en "chaotische" ademhalingspatronen. Omdat dit effect voorafgaat aan afname van de klinische symptomen en niet correleert met angst niveaus, zou de afname van respiratoire onregelmatigheid een direct effect van paroxetine op respiratoire funktie kunnen zijn. Dit ondersteunt de gedachte dat beinvloeding van de ademhaling een belangrijk mechanisme is van serotonerge anti-paniek medicatie, en dat een "normalisatie" van een dysfunctionerend ademhalingssysteem in PD een rol speelt.
Hoofdstukken 4 tot en met 7 richten zich op het experimenteel onderzoek van de ademhaling bij PD patiënten. De studie in hoofdstuk 4 illustreert dat patiënten met PD een hogere entropie in baseline ademhalingspatronen hebben dan gezonde controlepersonen, wat wijst op meer onregelmatigheden en complexiteit van de ademhaling. Dit kenmerk wordt niet beinvloed door angst-toestand of ernst van de ziekte. Entropie beschrijft de mate van structuurloosheid in processen en systemen. De hogere entropie in de ademhaling van PD patiënten kan wijzen op een intrinsieke instabiliteit van de respiratoire homeostase, waarop verschillende kritieke inputs kunnen werken als "verstorende" factoren, leidend tot paniekaanvallen. Om de hypothese te toetsen dat een hogere entropie in de ademhaling van PD patiënten een kwetsbaarheidsfactor voor paniek zou vormen, werd in hoofdstuk 5 onderzocht of de baseline respiratoire instabiliteit ten grondslag zou kunnenliggen aan de overgevoeligheid voor hypercapnie bij PD patiënten. De resultaten toonden aan dat patiënten die een paniekaanval kregen na een 35% $CO_2$ inhalatie een hogere baseline respiratoire onregelmatigheid hadden dan patiënten die geen paniekaanval kregen en gezonde controlepersonen. Ook bleek dat een hogere onregelmatigheid van tidal volume voorspellend is voor geinduceerde paniek. Deze resultaten ondersteunen het idee dat overgevoeligheid $CO_2$ gerelateerd is aan een dysfunctioneren van het ademhalingssysteem. Baseline chaotische ademhaling zou het vermogen van PD patiënten om adequaat te reageren op verstoringen, zoals een $CO_2$ inhalatie, beinvloeden en mogelijk het optreden van geinduceerde paniekaanvallen beinvloeden. Deze bevindingen zijn consistent met de resultaten uit hoofdstuk 6, waarin verschillende soorten van dyspnoe veroorzaakt door $CO_2$ inhalatie in PD patiënten patiënten werden onderzocht. "Moeite met ademen", voortkomend uit het bewust ervaren van de spieractiviteit van de ademhalingsspieren was het meest opvallend gevoel van dyspnoe in $CO_2$ geinduceerde paniekaanvallen. Deze associatie kan verklaard worden door de afwijkingen die in de ademha-

ling van PD patiënten worden gevonden. De toegenomen onregelmatigheid en instabiliteit in baseline ademhalingspatronen zou de mechanische efficientie van respiratoire responsen kunnen doen afnemen, en leiden tot een gevoel van "moeite met ademen" als zich respiratoire stimuli voordoen zoals bij de $CO_2$ inhalatie. Het "gevoel te stikken", voortkomend uit de activatie van chemoreceptoren lijkt ook betrokken te zijn in de $CO_2$ reactiviteit, maar lijkt niet centraal te staan bij onverwachte paniekaanvallen.
Het dagelijks roken wordt gerelateerd aan een verhoogd risiko op het ontstaan van paniekaanvallen en PD, mogelijk door het aantasten van het ademhalingssysteem. In hoofdstuk 7 onderzochten we de mechanismen die roken en het rspiratoir systeem in PD met elkaar verbindt. Patiënten die roken hebben een hogere baseline onregelmatigheid dan niet-rokende patiënten, terwijl rokende en niet-rokende gezonde controlepersonen niet verschilden in hun ademhalingspatroon. De hogere respiratoire onregelmatigheid in rokende patiënten en het ontbreken van een effect van roken op het ademhalingspatroon van gezonde vrijwilligers, kan wijzen op een specifieke invloed van roken op kwetsbare respiratoire funktie in PD. Aandoeningen van de luchtwegen waren niet verantwoordelijk voor de invloed van roken op de onregelmatige ademhaling, wat een direct negatief effect van roken op de ademhaling van PD suggereert. Tenslotte, de bevinding dat ook niet-rokende PD patiënten een onregelmatiger ademhaling hebben dan gezonden ondersteunt het idee dat de irregulaire ademhaling een intrinsiek kenmerk van PD patiënten kan zijn. Roken kan dan een "verstorende" factor zijn, specifiek op de baseline respiratoire instabiliteit van PD patiënten, wat mogelijk het onstaaan en/of voortbestaan van de aandoening beinvloedt.

Globaal genomen wijzen de resultaten uit de in dit proefschrift beschreven onderzoeken op een centrale rol van de ademhaling in de pathofysiologie van PD, en ondersteunen de gedachte dat paniekaanvallen specifiek gerelateerd zijn aan een dysfunctionerend ademhalingssysteem, meer dan slechts een equivalent van angst/stress reacties. De belangrijkste conclusies zijn dat 1) de baseline respiratoire entropie zou kunnen wijzen op dysfunctie van het ademhalingssysteem en zo een kwetsbaarheidsfactor voor paniek vormen. Instabiliteit in het respiratoir systeem zou kunnen leiden tot paniek als het systeem niet meer de externe/ interne stimuli kan hanteren en een evenwicht niet meer kan herstellen; 2) het verbeteren van de respiratoire dysfunctie /instabiliteit zou een specifiek aangrijpingspunt van anti-paniek

medicatie kunnen zijn. Deze hypothese zou getoetst moeten worden door de relatie tussen de modulatie van het respiratoir systeem op korte en lange termijn anti-paniek behandeling en klinisch resultaat.

Desalniettemin, enkele centrale punten staan nog open ter verheldering:

1) De bron van de respiratoire entropie kan uit meerdere mechanismen ontstaan. a) De respiratoire entropie kan een indicatie zijn van primaire respiratoire afwijkingen. Het kan een compensatoire respons zijn op dysfunctionele respiratoire signalen, zoals chemoceptieve input, of voortkomen uit een intrinsiek verstoorde activiteit in het respiratoir netwerk van de hersenstam dat vorm geeft aan de ademhalingspatronen; b) de respiratoire entropie zou verklaard kunnen worden door verstoringen van andere basale systemen. Recent onderzoek heeft een verstoorde cardiovasculaire en evenwichts systemen in patiënten met PD aangetoond, wat een algemener dysfunctie van fysiologische homeostase-regulerende neuronale circuits in PD veronderstelt; c) de respiratoire entropie kan voortvloeien uit hersengebieden hoger dan de hersenstam, zoals het limbisch systeem dat betrokken is in ademhalingsveranderingen tijdens emotionele toestanden. Hoewel onze studies geen effect van angst niveau op baseline respiratoire entropie aantoonden, kan een mogelijke invloed van de emotionele staat op ademhalingspatroon niet geheel worden uitgesloten. Verder onderzoek naar respiratoir/cardiaal/vestibulair geintegreerde patronen onder verschillende experimentele condities zou hier verheldering kunnen brengen.

2) De vraag of een hogere respiratoire entropie een "state" kenmerk van PD zou zijn of een "trait" marker voor de kwetsbaarheid voor PD. Verder familie-onderzoek is nodig om dit crucial punt op te helderen.

3) De mogelijke specifieke neuro-anatomische circuits die betrokken zijn in "respiratoire paniek".

Neurale kaarten, die het interne milieu van het organisme representeren, worden per moment verzameld in het Centraal Zenuw Stelsel, om de aanpassing van het organisme aan interne/externe veranderingen of dreigende toekomstige gebeurtenissen, teneinde het welzijn van het lichaam te behouden. Deze basale regulatoire processen vinden continu plaats, onbewust en dringen slechts nu en dan door tot het bewustzijn als "primaire emoties". Dergelijke emotionele toestanden, zoals extreme dorst of ademnood, kunnen de evolutionaire rol van het signaleren van bedreigde overlevingskansen hebben. Er zijn aanwijzingen dat primaire

emoties gedragen worden door gelijkaardige circuits in phylogenetisch oude hersengebieden. Recente brain-imaging studies hebben de activatie/deactivatie patronen gedurende 5% en 8% $CO_2$ geinduceerde ademnood onderzocht in gezonde proefpersonen. Het bewustzijn van de respiratoire gewaarwordingen behelsde een breed netwerk van activatie in de "primitieve" hersengebieden inclusief de hersenstam, mesencephalon, hypothalamus, limbische en paralimbische gebieden, hippocampus en parahippocampale en fusiforme gyrus, insula anterior, cingulate anterior en cerebellum, terwijl deactivatie in de dorsale en posterior cingulate en prefrontale cortex gevonden werd. Deze hersenregionen behelsen ook gebieden die niet betrokken zijn in angst modellen, zoals het cerebellum, en zijn niet volledig overlappend met het angst-netwerk circuits. Deze gegevens steunen de gedachte dat paniekaanvallen verschillen van angst-reacties en misschien een uiting zijn van "oer" emoties voortkomend uit soecifieke phylogenetisch oude hersen circuits die fysiologische waarnemingen/sensaties verwerken die gerelateerd zijn aan homeostatische functies. Klein's "false suffocation alarm" zou tot het spectrum van oer-emoties kunnen behoren. Een afwijkend verwerken van informatie betreffende het organismes interne milieu in het netwerk dat tengrondslag ligt aan basale emoties, kan leiden tot een afwijkend "respiratoire oer-emotie" dat het bewustzijn van de patient betreedt als "paniek aanval". De respiratoire onregelmatigheid en mogelijk abnormale signalen van cardiale en vestibulaire systemen kunnen triggers zijn voor het activeren van deze circuits in PD. Echter, dit blijft speculatief daar de beschikbare data nog onvoldoende zijn. Verder onderzoek middels brain imaging zou ruimte kunnen bieden om deze hypothesen te toetsen.

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## Curriculum vitae

Daniela Caldirola was born in 1965 in Milan, Italy. She graduated from Berchet High School of Classical Studies in Milan. She obtained her Medical Degree from the State University of Milan, School of Medicine. She was a clinical fellow in psychiatry, from 1995 to 1999, under the supervision of Prof.dr. Laura Bellodi, director of the Anxiety Disorders Clinical and Research Unit, San Raffaele Hospital in Milan. In 1999 she obtained the Specialization in Psychiatry from the State University of Milan. Since 1999, she has worked as a psychiatrist, at the Anxiety Clinical and Research Unit, San Raffaele Hospital, Milan. Since 2002, she has been an Assistant Professor in Psychiatry, at the Vita-Salute University School of Psychiatry, Milan. She has recently received a four-year grant for research on the biological basis of panic disorder.